

ZOOLOGY

HIGHER SECONDARY FIRST YEAR

Vol. II

TAMILNADU TEXTBOOK SOCIETY

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Higher Secondary – First Year

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CHAPTER XV

Urinogenital System

The urinogenital system is a composite organ system formed of two distinct organ systems namely an excretory or urinary system and a reproductive or genital system. Most of the fresh water fishes are ammonotelic though they excrete some urea. Elasmobranchs, amphibians and mammals are ureotelic animals since they excrete their nitrogenous wastes as urea. The reptiles and birds are uricotelic in their nitrogen metabolism. They excrete uric acid as their nitrogenous waste product. The genital system is concerned with the production of gametes namely the eggs, the sperms and thus enables the continuance of the race.

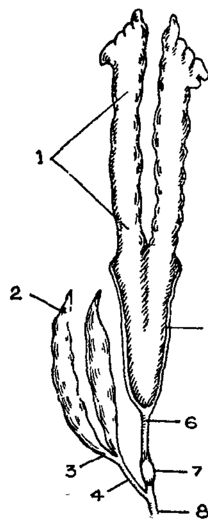


Fig. 125 Mugeil Male Urinogenital System

1. Kidney 2. Testis
3. Vas deferens
4. Common vas deferens 5. Ureter
6. Common ureter
7. Urinary bladder
8. Urino-genital opening

A. EXCRETORY SYSTEM

MUGEIL

It is composed of paired kidneys, paired ureters, an urinary bladder and an urinogenital sinus opening to the outside as the urinogenital pore. The kidneys are of the mesonephros type. They are present dorsal to the airbladder and ventral to the vertebral column in the trunk cavity. The kidneys are elongated strap shaped structures and are fused at their posterior ends to form a single unit. Each kidney is divisible into anterior non-renal part and a posterior functional excretory portion. The ureters arise from the lateral border of the posterior end of each kidney and they unite behind to form a single common ureter. It is enlarged at its posterior end to form the urinary bladder. Behind the urinary bladder it joins a

common vas deferens to form the common urinogenital duct. It opens into the urinogenital sinus which opens to the outside as the urinogenital pore behind the anus.

FROG

It is composed of paired kidneys, paired ureters and an unpaired urinary bladder. The kidneys are compact structures and are meso-

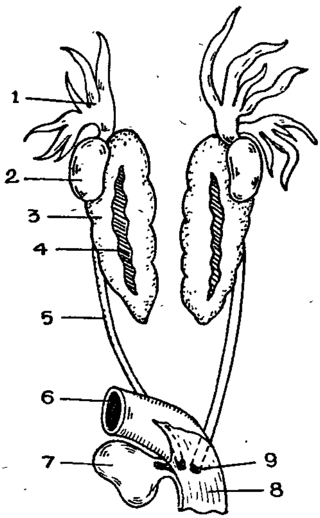


Fig. 126

Frog—Male Urinogenital System

1. Fat body 2. Testis 3. Kidney
4. Adrenal body 5. Ureter
6. Rectum 7. Urinary bladder
8. Cloaca 9. Opening of Ureter

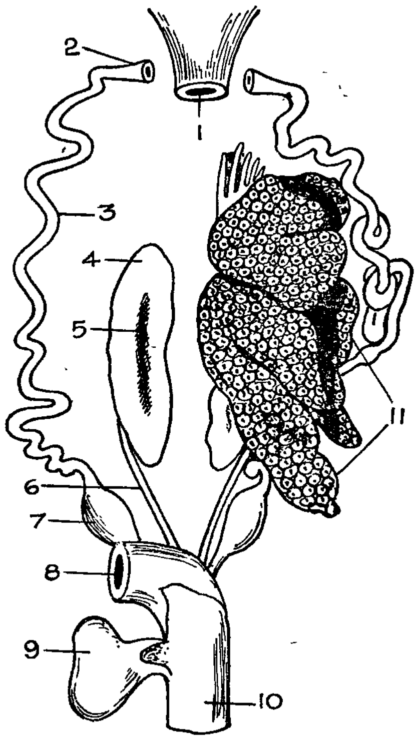


Fig. 127

Frog—Female Urinogenital System

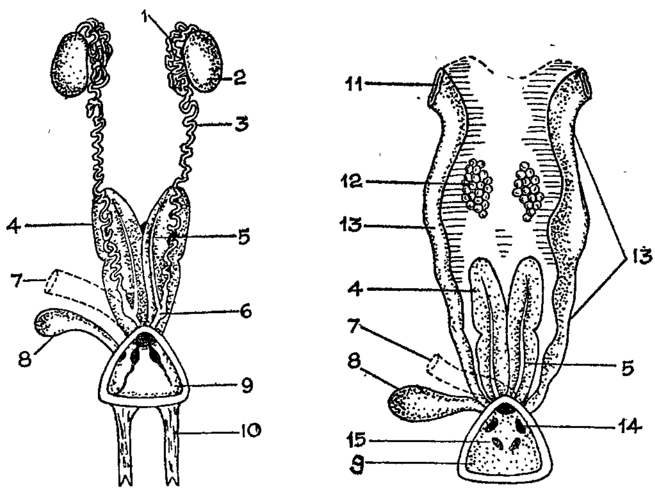
1. Oesophagus 2. Oviduca funnel
3. Ureter 4. Kidney 5. Adrenal body
6. Ureter 7. Ovisac 8. Rectum
9. Urinary bladder 10. Cloaca
11. Ovary

nephric. They lie in the middle region of the body cavity one on either side of median vertebral column. The male and female excretory systems are similar. Each kidney is dark red in colour and flat

in shape. It has an outer convex surface and an inner surface dividedinto three lobes by means of two constrictions. From the posterior outer margin of each kidney arises a white tubular duct termed the ureter which leads into the cloaca. Just at the place where the ureters open into the cloaca there is an unpaired thin walled sac, the urinary bladder. It is meant for storing the urine. Each kidney consists of a large number of (about 20,000) coiled glandular uriniferous tubules which are bound together by connective tissue. Each kidney tubule has a cup like Bowman's capsule containing blood capillaries (glomerulus) and a fine coiled renal tubule. The Bowman's capsule and glomerulus together constitute the Malpighian corpuscle. The urine is extracted from the blood and it is sent out through the cloaca.

LIZARD

It is composed of paired kidneys, paired ureters and an unpaired urinary bladder. The kidneys which are



Male Female

Fig. 128 Calotes—Urinogenital System

1. Epididymis 2. Testis 3. Vas deferens 4. Kidney
5. Ureter 6. Seminal vesicle 7. Rectum 8. Urinary bladder
9. Cloaca 10. Penis 11. Oviducal funnel 12. Ovary
13. Oviduct 14. Opening of oviducts 15. Opening of ureters.

metanephric lie at the posterior part of the body cavity one on each side of the vertebral column. They are

covered ventrally by peritoneum. Each kidney consists of two lobes an anterior and a posterior, which are separated by one lateral groove. The posterior lobes of the two kidneys are united. From the ventral surface of each kidney arises a delicate whitish duct the ureter. It runs towards the cloaca to open into it. The urinary bladder arises from the ventral wall of the cloaca. In males the ureter at its posterior end combines with the terminal end of the vas deferens to form a common urinogenital duct which opens into the cloaca. The lizards are uricotelic and they excrete their nitrogenous wastes in the form of solid ball of uric acid crystals. In the female the ureters open independently into the cloaca.

BIRD

It is composed of paired kidneys and paired ureters. Urinary bladder is absent. The kidneys are dark red in colour and are lod-

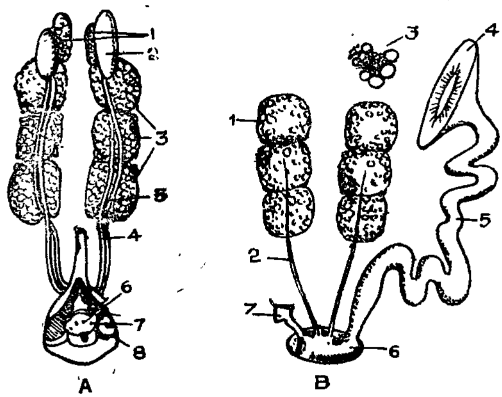


Fig. 129 Pigeon—Urinogenital System

A. Male
1. Epididymis 2. Testis 3. Kidney 4. Vas deferens 5. Ureter
6. Cloaca 7. Opening of ureter 8. Opening of Vas deferens
B. Female
1. Kidney 2. Ureter 3. Ovary 4. Oviducal funnel
5. Oviduct 6. Cloaca 7. Rudimentary oviduct

ged in the cavity of the pelvic region. Each kidney is composed of three lobes namely ananterior, a middle, and a posterior lobe. They are attached to the dorsal body wall and are covered by peritoneum. Histologically the kidney is composed of numerous uriniferous tubules. Each tubule is provided with a small glomerulus filtrate. From

the ventral surface of each kidney arises a delicate ureter which runs backwards to open into the cloaca. The male and female excretory systems are identical.

RAT

It is composed of paired kidneys, paired ureters and an unpaired urinary bladder. The kidneys are metanephric and abdominal in position. They are typically bean shaped and lie in the middle region of the abdominal cavity one on either side of the vertebral column. They are dark red in colour. The kidneys are not placed at the same level and so there is a certain amount of asymmetry. The right kidney is placed at a higher level than the left.

The outer margin of the kidney is convex while the inner margin facing the vertebral column is concave. The inner cavity is known as the hilus. A slender muscular tube the ureter

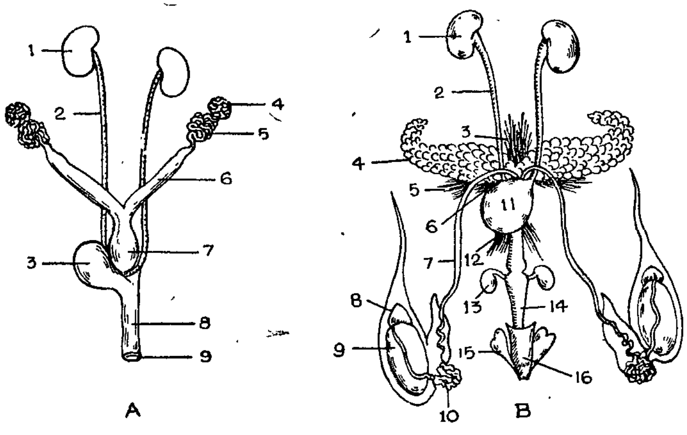


Fig. 130 Rat—Urinogenital System

A. Female

1. Kidney 2. Ureter 3. Urinary bladder 4. Ovary 5. Fallopian tubes 6. Uterus 7. Vagina 8. Vestibule 9. Vulva

B. Male

1. Kidney 2. Ureter 3, 5, 6. Ampullary glands 4. Vesicular glands 7. Vas deferens 8. Caput epididymis 9. Testis 10. Cauda epididymis 11. Urinary bladder 12. Prostate glands 13. Cowper's glands 14. Urethra 15. Preputial gland 16. Penis.

arises from the hilus and runs backwards to open into the urinary bladder which lies in the posterior part of the abdomen, ventral to the rectum. The ureter opens into the kidney at the

hilus by a wide opening called the pelvis and serves to conduct the urine from the kidneys to the urinary bladder. The urinary bladder is continued backwards by a narrow neck into the urinogenital canal and it passes through a muscular portion the urethra and opens out as the urinogenital aperture at the tip of the urethra or penis in the male.

A longitudinal section of the kidney reveals two distinct regions namely an outer dark red cortex and an inner pale red medulla. Histologically the kidney is composed of numerous microscopic units called the nephrons. Each nephron commences in the cortex in the form of a double walled cup called the Bowman's capsule enclosing in its cavity a network of blood capillaries referred to as glomerulus. The Bowman's capsule is continued into the medulla as a tubule called uriniferous tubule which runs in loops. A number of these tubules join together to form the collecting tubules. Several collecting tubules aggregate to form bundles called the pyramids which open into the pelvis.

B. REPRODUCTIVE SYSTEM

MUGEIL

Male: It consists of paired testes, paired vasa deferentia and a common vas deferens. Each testis is a bean shaped structure and these are present by the side of the kidneys. From each testis arises a tubular duct, the vas deferens. The vasa deferentia unite to form a common duct and it opens into the urinogenital sinus.

Female: It consists of a pair of ovaries and a pair of oviducts. The ovaries are sac like structures filled with eggs. Each ovray is continued as an oviduct. The two oviducts open into the urinogenital sinus. Mugeil is on oviparous form.

FROG

The male reproductive system consists of a pair of smal yellowish elongated bodies, the testes, attached to the antero-ventral surface of the kidneys by a narrow mesentery called the mesorchium. A number or small ducts the vasa efferentia, located in this mesentery connect each testis with the kidney. The testis is made up of a coiled mass of seminiferous tubules in

which the sperms are produced. Sperms formed in the seminiferous tubules pass into the kidney through the vasa efferentia. From the kidney the sperms are passed out through the ureter. The ureter in the male thus serves to carry both urine and the sperms. Hence it is known as urinogenital duct. Attached to the anterior end of each kidney is an yellowish, much branched fat body. The fat bodies function as fat storage organs. The fat stored is used up whenever necessary.

The female reproductive system consists of a pair of ovaries and oviducts. Each ovary is a much folded sac attached to the antero-ventral surface of the kidney by a narrow mesentery called mesovarium. The size of the ovary varies according to the season. During the breeding seasons the ovary is large, filled with numerous rounded eggs. The eggs are liberated from the ovaries into the body cavity from where they enter the oviducts through the oviducal funnels. The oviducts are highly coiled whitish tubes suspended from the dorsal ody wall by mesenteries. The opening of the oviducts namely the oviducal funnels are located near the base of the lungs one on either side of the oesophagus. Anteriorly the ducts are thin walled and dilated. As the eggs pass down the oviducts they are coated with layers of albumen or jelly. The terminal part of the oviduct is dilated into an ovisac, where the eggs are collected before they are discharged to the outside through the cloaca. Fat bodies occur as in the male.

LIZARD

The male reproductive system consists of paired testes, paired epididymis, paired vasa deferentia and paired penis. The testis are oval in shape and more or less whitish in colour. They are attached to the dorsal body wall in the middle region of the body cavity by means of the mesorchium. From each testis arises several delicate vasa efferentia and they open into the epididymis. The epididymis is continued as the vas deferens. It joins at its posterior end with the ureter to form the urinogenital duct, which opens into the cloaca. Inside the cloaca a pair of grooves run into copulatory structures, penes, which project out as a pair of muscular structures on either side of the cloaca. The penis acts as the intromittant organ helping in the transference of sperms into the cloaca of the female.

The female reproductive system consists of paired ovaries and oviducts. The ovaries are situated on either side of the vertebral column attached by the folds of peritoneum. Each oviduct enlarges at its anterior end in the form of a wide funnel shaped structure termed the oviducal funnel, and at its posterior end it opens into the cloaca. The wall of the oviduct is highly glandular and it secretes albumen and a tough membrane. Fertilization takes place in the anterior part of the oviduct before the shell is formed. The lizards are oviparous and they lay numerous small eggs.

BIRD

The male reproductive system in bird consists of paired testes, paired vasa deferentia and paired vesicula seminalis. The testis is oval in shape and is attached to the anterior part of the kidney by mesorchium. The size of the testis varies according to the seasons. From the testis arises the vas deferens. At its posterior end it is dilated into the seminal vesicle for storing the sperms. The vas deferens opens into the urodaeum of the cloaca. The wall of the cloaca is eversible. Copulatory organs are absent.

The female reproductive system in bird is characterised by the conspicuous absence and the degeneration of right ovary and right oviduct. Sometimes rudimentary ovary and remnants o oviduct may be present. These adaptations serve to reduce the weight of a bird which is a positive advantage in flight. The left oviduct is enlarged at the anterior end to form the left oviducal funnel opening into the body cavity. Following the oviducal funnel is a coiled glandular middle part which secretes albumen around the egg and a posterior short portion which secretes the egg shell. The oviduct at its posterior end opens into the urodaeum of the cloaca. Birds are strictly oviparous. The eggs are heavily yolked and provided with a thick porous shell.

RAT

Male: The reproductive organs of male rat consist of a pair of ovoid testes which in the young animal are situated close to the dorsal wall of the abdominal cavity near the kidneys. As the rat becomes sexually mature the testes descend into a pair of pouches of the body wall called the scrotal sacs situated

one on each side of the base of the penis. The cavities of each scrotal sac communicates with the abdominal cavity by a narrow passage the inguinal canal. From the lower end of each testis arises a much coiled tube called the epididymis which is continued as the vas deferens. Each vas deferens passes through the inguinal canal into the abdominal cavity, loops around the ureter of its side and runs back to open into a median sac the uterus masculinus which opens into the urethra The urinary bladder is continued posteriorly as the uinogenital canal or the urethra which traverses the penis to open at its tip by the urinogenital aperture. The penis is made up a spongy tissue containing plenty of nerves and blood vessels. The tip of the penis is in the form of a swollen mass of erectile tissue, the glans penis, which is surrounded by a loose retractile fold of the skin known as the prepuce. Associated with the male reproductive organs are a number of accessory glands and these are (i) the prostate glands which lie close to the uterus masculinus opening by several ducts into the urinogenital canal. (2) a pair of Cowper's glands lying beind the prostate glands the secretions of which contribute to the spermatic fluid and render the sperms more active. (3) a pair of vesicular glands at the base of the urethra and their ducts open into it (4) a pair of small ampullary glands encircle the vas deferens, and their ducts open into the uterus masculinus or urethra (5) a pair of preputial glands. (6) a pair of rectal glands. (7) a pair of perineal glands which lie at the sides of the penis.

Female : The ovaries consist of a pair of small ovoidal structures attached to the dorsal wall of the abdominal cavity behind the kidneys. Each ovary has on its surface a number of projections known as the Graafian follicles each of which contains an ovum.

A pair of oviducts is present, each of which is differentiated into an anterior fallopian tube which opens into the body cavity by a wide funnel shaped opening and a posteior enlarged uterus. The uteri unite to form a median tube called the vagina. The vagina joins the neck of the urinary bladder whicl. is continued behind as the urinogenital canal or the vestibule which in turn opens out by the urinogenital aperture ventral to the anus.

On the ventral wall of the vestibule lies the clitoris, a small hard prominence which is the homologue of penis. On the dorsal wall of the vestibule are present two small Cowper's glands. Perineal and rectal glands are present as in the male. Prostate glands are absent in the female. The rat is a viviparous form. Eight to ten young ones are born at a time. These are at first suckled by the mother but later become herbivorous.

CHAPTER XVI

Sense Organs

The awareness of an organism to the various changes of the environment is due to the presence of sense organs. The sense organs are composed of sensory cells or receptors. The receptor cells occur almost everywhere in the body. Different types of receptors exist in animals for detecting the various stimuli. The more common types of receptors met with in animals are a follows:

Stimulus	Nature of receptors
smell	Olfactoreceptors
Taste	Gustoreceptors
Sight	Photoreceptors
Hearing	Phonoreceptors
Touch	Tangoreceptors
Temperature	Thermoreceptors
Pain	Algesireceptors
Water current	Rheoreceptors
Equilibrium	Statoreceptors

A. ORGANS OF SIGHT (EYE)

MUGEIL

Organs of sight in fishes are the eyes. They are located in the orbits and are directed laterally. The eye is relatively large in size and is elliptical in form having short antero posterior axis. It is a hollow structure and its wall is composed of three coats. There is an outer fibrous, tunic a middle uvea and retina. The outer fibrous tunic is thick and tough. It protects the eye ball, and maintains its form. It has two distinct regions, namely a small anterior transparent cornea, a large posterior opaque sclerotic coat. This sclerotic coat is cartilagenous and it receives all eye muscles. The cornea is composed of a

special type of connective tissue. It has an outer covering of transparent epithelium called the conjunctiva. The middle coat or the uvea has three distinct regions namely the iris, the ciliary body and the ciliary process. The retina contains the rods but lacks the cones. The cornea is flat. The lens is large and rounded in shape. The outer part of the choroid coat has a specialised silvery layer termed as argentea. It is composed of a reflective chemical namely guanine. At the entrance of the optic nerve there is a mass of blood vessels termed as the choroid gland. There is a thick strand of vascular tissue which is formed as a fold of the choroid called the falciform process, and it extends from the entrance of the optic nerve to the posterior end of the lens. Here it ends as a knob like structure termed as the companula halleri. It is supposed to help in the focussing of lens. The eyelids are absent, in some species adipose eyelids are present.

FROG

The organs of sight are located in the orbits. Each eyeball is spherical in shape, and is moved by a set of six muscles, attached to its outer surface. The outer covering of the eyeball is tough and called the sclerotic coat. Anteriorly this is modified to form the transparent cor nea. Underneath this coat is the choroid coat which is pigmented and vascular with an opening in front of the pupil. The pigmented front portion of the choroid is known as the iris which is perforated by the pupil. The pupil contracts or regulate the amount of light entering the eye. Behind the pupil is a spherical crystalline lens. The lens does not change its shape or position so that the eye can form images of objects lying only at certain distance. The lens is held in position by ciliary processes

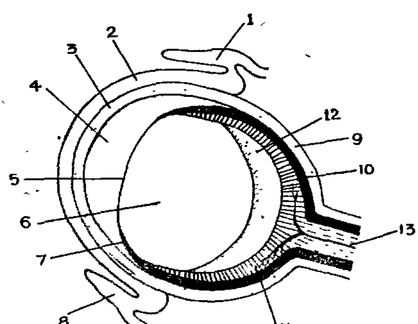


Fig. 131
Frog-Eye
1. Upper eyelid 2. Conjunctiva
3. Cornea 4. Aqueous humour 5.P upil
6. Lens 7. Iris 8. Lower eyelid 9. Sclerotic coat 10. Choroid layer 11. Retina
12. Vitreous humour 13. Optic nerve

which extend from it to the choroid wall. The innermost coat is the retina containing the sensory rods and cones which are the receptors of light stimuli. The space in front of the lens, the anterior chamber, contains a fluid the aqueous humour and that behind (posterior chamber) a jelly like vitreous humour, both serving to nourish and maintain the form of the eyeball.

LIZARD

The eye of lizard is more advanced than that of the fish and frog in order to function on land. Both eyelids are immovable. There is a third eyelid namely the nictitating membrane. Distinct harderian glands and lacrymal glands are present and their secretions lubricate the eyeball and serves to keep the surface of the eye moist. As in the fish the eyeball consists of 3 coats namely an outer fibrous tunic, a middle uvea and an inner retina. In the other details the eye resembles that of the frog. Accomodation is brought about as in mammals by altering the focal length of the lens through a change in its concavity. This in turn is caused by the contraction of the ciliary muscles which squeeze the lens making its anterior surface more convex.

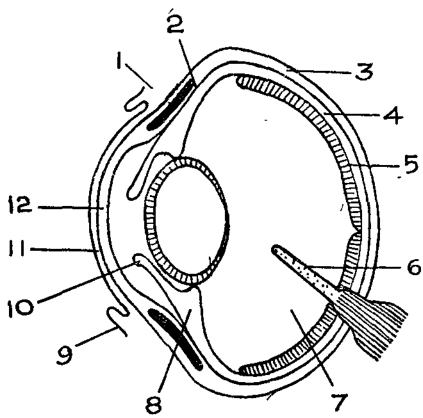


Fig. 132
Calotes—Eye

1. Upper eyelid 2 & 3. Sclerotic coat
4. Choroid layer 5. Retina 6. Pecten
7. Vitreous humour 8. Ciliary muscles
9. Lower eyelid 10. Iris 11. Conjunctiva
12. Cornea

BIRD

The eye of bird is very well developed. It is due to the keen sense of sight of the bird. The eye resembles that of the reptiles in its basic structure.

The eye is very large in proportion to body size. It has movable upper and lower eyelids. There is a third nictitating membrane. Harderian gland lubricates the nictitating membrane

The secretions of the lacrymal glands serve to keep the eye moist, removes the dust form the corneal surface and provides nourishment to the non vascular cornea.

The eyeball is flattened antero posteriorly being bi-convex in form. The wall of the eye ball is formed of three coats namely fibrous tunica middle uvea and the inner retina.

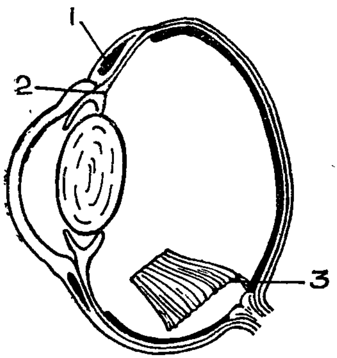


Fig. 133
Bird—Eye

1. Sclerotic coat 2. Ciliary organ 3. Pecten

The eye of birds is characterised by the presence of a peculiar soft, vascular, rectangular, pleated blackish body the pecten projecting into the vitreous humour from the blind spot. Various functions are attributed to it. (1) since it is erectile it is believed to exert a pressure on the lens to alter its curvature during accommodation. (2) it is believed to help in the perception of movement of objects by casting its shadow on the retina. (3) It is believed to act as a nourishing centre in view of its rich vascularization.

Accommodation is brought about by the striated ciliary muscles.

RAT

Eyes are lodged in eye sockets or orbits. The eyeball is roughly spherical in form. It resembles basically the structure of the eye of a bird except for the absence of pecten.

The eyelids are supported by a single row of eyelashes. This feature is characteristic of mammals. The eyes of mammals in general have a good power of accommodation.

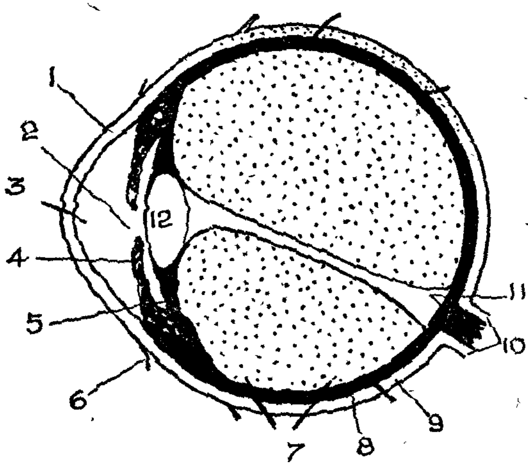


Fig. 134 Rat—Eye

1. Cornea 2. Pupil 3. Aqueous chamber 4. Iris 5. Ciliary Process 6. Conjunctiva 7. Retina 8. Pigment coat 9. Sclerotic coat 10. Optic nerve 11. Blind spot 12. Lens

B. ORGANS OF HEARING (EAR)

The *organs concerned* with hearing are the ears. In the vertebrates the ear is composed of 3 regions namely the inner ear, the middle ear and the external ear. The inner ear and the middle ear are present in the amplibians, reptiles, birds and mammals. The external ear is found only in the mammals. The ear in *vertebrates has two functions namely auditory and static.* The inner ear is composed of a membranous labyrinth and the bony labyrinth. The membranous labyrinth basically consists of two sacs namely a dorsal longer sac the utriculus and a ventral sac the sacculus and these two sacs are demarcated by a definite constriction. The sacculus has a small outgrowth termed as the *lagena which increases in its length and in the mammals* it becomes spirally coiled forming the cochlea containing the organ of Corti-chief organ concerned with hearing. With the utriculus are connected 3 tubes called from their form

the semicircular canals. They open by both ends into the utriculus. Of the three semicircular canals one is horizontal while the other two are vertical and at right angles to each other. Each canal has at one end a dilated bulb or ampulla. The membranous labyrinth is filled with a fluid the endolymph. The space between the membranous labyrinth and bony labyrinth is filled with a fluid termed the perilymph. It serves to absorb the shocks and protects the membranous labyrinth. The middle ear in the vertebrates is composed of the tympanum, tympanic cavity and the ear-ossicles. The amphibians, reptiles and birds have only a single ear ossicle termed the columella, while in the mammals a chain of three ossicles are present termed the malleus, incus and stapes. The external ear in the mammals is composed of an external auditory meatus and a cartilagenous pinna or external earlobe. The pinna is meant for focussing the sound waves into the middle ear. The presence of pinna is characteristic of mammals.

MUGEIL

In the fish only the inner ear is present. The middle and external ears are absent.

The sound waves are transmitted through the operculum or gill slits to the membranous labyrinth. The inner ear is delicate but complex in structure and it is termed as the membranous labyrinth. It is enclosed in the auditory capsule of the cranium. The auditory capsule in termed as the bony labyrinth. There is a narrow space between the membranous and bony labyrinth termed the perilymphatic space and it is filled with a fluid perilymph.

The membranous labyrinth has a laterally compressed sac the vestibule. It is partially divided into two chambers the upper narrow utriculus and the lower wide sacculus. The two chambers communicate with one another by a wide passage the sacculo-utricular duct. The latter becomes long and narrow in higher forms.

The sacculus gives rise to a short projection termed as the lagena. A narrow canal arises from the dorsal side of the sacculus and runs upwards. It is termed as the ductus endolymphaticus. It does not open to the exterior. Three semicircular ducts are

attached to the utriculus. These are placed at right angles to each other. One of them is horizontal while the other two are vertical. They open into the utriculus. One end of the tube is enlarged to form the ampulla.

In the mugeil the sacculus is large and it has solid calcareous bodies termed the otoliths. These are constant in number for each species. The sensory patches of the ampullae are called the cristae ampullares or cristae acousticae. While those of the utriculus sacculus and lagena are termed the maculae acousticae. The cristae and maculae are composed of neuro sensory and supporting cells.

The cavity of the membranous labyrinth is filled with a fluid termed the endolymph. The epithelium is sensitive to the movement of the endolymph in the canals. When the equilibrium is changed the movements of the endolymph starts the impulses which are carried to the brain by the auditory nerve.

FROG

The organs of hearing are a pair of ears which consist of the middle ear and the internal ear. The external ear is absent in Frog. The middle ear is closed externally by the tympanic membrane (ear drum) which receives sound waves from air and water. Vibrations induced in the tympanic membranes are transmitted across the cavity of the middle ear by a rod shaped columella, connecting the ear drum and the inner ear. The inner ear is a complicated structure known as the membranous labyrinth which lies within the auditory capsule. The membranous labyrinth consists of an irregular sac like portion known as vestibule and 3 semicircular canals. The vestibule inturn is divided into an upper larger portion called the utriculus and a lower smaller portion called the sacculus. The three semi circular canals arise from the utriculus. One of the semi circular canals is horizontal and the other two of the semi circular canlas are termed as the anterior and posterior semicircular canals. One end of each is enlarged to form the ampulla. The auditory cavity contains a fluid the perilymph, while the membranous labyrinth contains a fluid called the endolymph and it bears the sensory nerve endings of the auditory nerve. Sound waves transmitted to the inner ear cause the endolymph to vibrate and these vibrations are registered by the sen-

sory end organs of the auditory nerve. The function of maintenance of equilibrium is performed by the semicircular canals which are filled with fluid. The pressure of this fluid stimulates certain nerve endings to carry the impulses along a branch of the

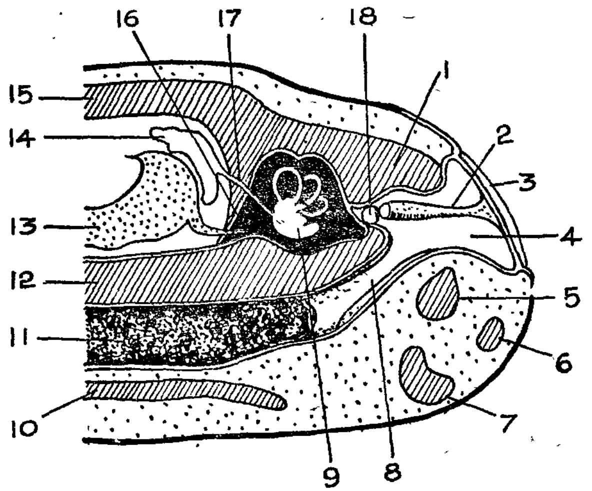


Fig. 135 Frog—Ear

1. Proatic bone 2. Columella 3. Tympanum 4. Middle ear 5. Pterygoid 6. Quadrato-Jugal 7. Angulo splenial 8. Eustachian tube 9. Lagena 10. Hyoid 11. Pharynx 12. Parasphenoid 13. Brain 14. Endolymphatic sac 15. Fronto parietal 16. Auditory nerve 17. Endolymphatic duct 18. Stapes

auditory nerve to the brain, where they are intrepreted as the sensations of equilibrium. Thus the membranous labyrinth serves the double function of hearing and the maintenance of equilibrium.

LIZARD

The ear of lizard is composed of external auditory meatus, middle ear and the inner ear. The external auditory meatus consists of a small, vertically oval shallow pit situated behind the eye. It has at its bottom an oval patch of skin termed the tympanum.

Middle ear: It consists of an air filled cavity called the tympanic cavity, which is bounded internally by the auditory capsule and externally by this tympanum. From the lower median part

of the tympanic cavity a narrow passage the Eustachian tube extends downwards and inwards to open into the posterior part of the pharynx. A slender rod like ear ossicle the columella aurcs stretches arcoss the tympanic cavity.

Internal ear: It is similar to that of frog.

BIRD (Columba livia)

The ear of bird is similar to that of the mammal except that it lacks pinnae and its cochlea is not coiled.

External ear: It begins as a small aperture, the ear opening, situated on the side of the head a little behind the eye. It is surrounded by small auricular feathers and leads into a short tube, the external auditary meatus. The latter is closed internally by a thin skin, tympanum. The middle ear is similar to that of the frog and reptile.

Internal ear: It is similar to that of frog and the lizard except for the sacculus. The sacculus is prolonged into a long curved tube the cochlear duct. The part of the bony labyrinth surrounding the cochlear duct is called the cochlear canal. The cochlear duct and the cochlear canal are together referred to as the cochlea. The cochlear duct is joined to the cochlear canal on the sides, but is free above and below with the result, the cochlea shows in its transverse section three chambers: the upper scala vestibuli, the middle scala media and the lower scala tympani. The scala media is in reality the cochlear duct, and being an extension of the sacculus, contains endolymph. The floor and the roof of the scala media are respectively called the basilar membrane and the Reissnar's membrane. The basilar membrane

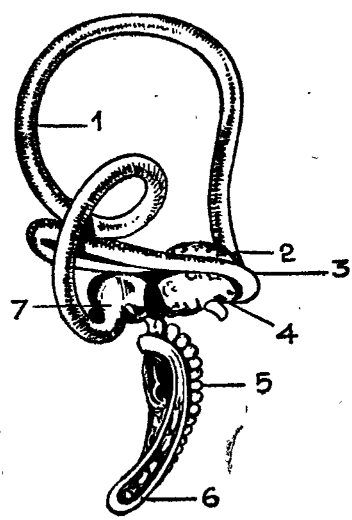


Fig. 136
Bird—Ear

1, 3, Semi circular canal
2. Utriculus 4. Fenestra
5, 6. Cochlea 7. Ampulla

bears the essential organ of hearing called the organs of Corti. It consists of tall auditory cells bearing auditory hairs on the free ends and nerve fibres at the other ends. The nerve fibres join to form the cochlear branch of the auditory nerve. A gelatinous tectorial membrane overlies the auditory hair of the organ of Corti. The organ of Corti perceives sounds of higher frequencies. A sensory spot, called macula lagena, at the tip of cochlear duct perceives sounds of lower pitch. The scala vestibuli and scala tympani contain perilymph and are continuous with one another at the tip of the cochlea by a passage called helicotrema. Sensory spots of the ampullae are called the cristae ampullares and those of the vestibule are termed the maculae. There is one crista in each ampulla. There is one macula in the utriculus and one in the sacculus. These are respectively called macula utriculi and macula sacculi. The cristae and maculae are patches of auditory cells with auditory hair and nerve fibrils. The latter join to form the vestibular branch of the auditory nerve. A gelatinous mass, the cupula overlies the auditory hair. It contains tiny calcareous particles, the otoconia, in it. The cristae ampullares are perhaps concerned with the sense of direction and equilibrium.

RAT

The ear is the organ of hearing and maintenance of equilibrium of the body and it attains the highest degree of development in the mammals. It is made up of three parts namely (i) the external ear (ii) the middl ear and (iii) the internal ear. The external ear consists of an expanded portion called the pinna or the earlobe and a tubular passage the auditory tube or the external auditory meatus leading to the tympanum or the ear drum. The pinna or the ear lobe is supported by cartilage and it serves to collect sound vibrations. The tympanum which is stret-

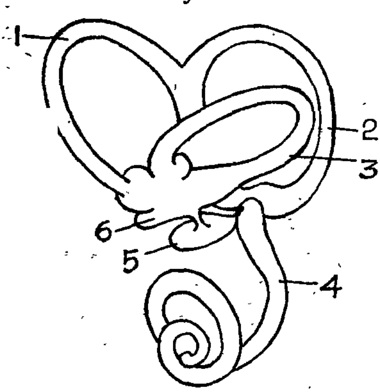


Fig. 137 Rat—Ear

1, 2, 3. Semicircular canals 4. Cochlea 5. Sacculus 6. Utriculus

ched across the inner end of the external auditory meatus marks the beginning of the middle ear. Extending across the cavity of the middle ear from the tympanum to a membrane covered aperture on the body wall of the inner ear is a chain of three bones called the ear ossicles. The ear ossicles beginning from the tympanic end are the malleus, the incus and the stapes. The malleus is hammer shaped, the incus is anvil like, and the stapes is in the form of a stirrup. These ossicles serve to conduct sound vibrations from the tympanum to the inner ear. The inner ear consists of two parts namely the membranous labyrinth and the bony labyrinth. The bony labyrinth encloses a cavity having the same shape as that of the membranous labyrinth. The membranous labyrinth lies in this cavity and the narrow space between it and the bony labyrinth is filled with a fluid the perilymph to which the vibrations of the tympanum are conveyed by the ear ossicles through the fenestra ovalis. The membranous labyrinth consists of 3 semicircular canals one horizontal and two vertical and a vestibule with two sacs known as the utriculus and sacculus followed by a spirally coiled structure the cochlea. One end of the each semi ciruclar canal is dilated to form an ampulla. Patches of sensory cells are present in side the ampullae and inside the utriculus and sacculus which are innervated by a branch of the auditory nerve and which serve in the maintenance of equilibrium. The cochlea which is spirally coiled contains the organ of hearing called the organ of corti. The vibrations of the perilymph make their exit through the fenestra rotunda present in the bony labyrinth behind the fenestra ovalis. The cavity of the middle car communicates with the pharynx through the eustachian tube. It serves to ecualise the pressure on both sides. Hearing will be normal only if the pressure is equal on both sides of the tympanic membrane.

CHAPTER XVII

Organization of the Cell

Living matter is called protoplasm. Typically it is more or less viscous, trasparent and is a complex mixture of many substances. It may contain materials which may be unnecessary for life and other nonliving substances such as fat or starch particles. But, whenever the term protoplasm is used, it refers to a unit of matter which manifests the characteristics of life.

Protoplasm is typically maintained in units called cells which are the basic organizational units of life. The cell can be defined as the biological unit of activity. It is the smallest portion of the organism that exhibits the range of properties associated with living matter. It is delimited by a semi-permeable membrane and is capable of self-reproduction in a medium free of other living systems. Some animals and plants consist of only one cell (unicellular). A great majority of the organisms are multicellular.

Early History

The early history of cell science is intimately bound to the development of optical lenses, and their combination in the construction of the compound microscope. The credit for the discovery of the cells goes to Robert Hooke (1665) who, while investigating the texture of cork by means of a magnifying lens, found certain structures with empty cavities which he called by the name of 'cells'. Hooke's observations were quickly followed by those of Malpighi and Grew which clarify to some extent the observations of Purkinje, Leuwenhoek and others.

Further progress in the study of cell science came in the beginning of the 19th century when Schleiden (1838) and Schwann (1839) formulated the cell theory. The recognition of the cell and the formulation of the cell theory are among the important discoveries of man. The theory states that "cells are organisms,

and entire animals and plants are aggregates of these organism arranged according to definite laws." This statement cannot be bettered today.

The immediate consequence of this theory was the establishment of facts such as

(1) that every cell is formed by the division of another cell (Virchow 1858).

(2) That there are fundamental similarities in the chemica composition and metabolic activities of all cells though they may be specialised for different functions and

(3) that the function of the organism as a whole is a result of the sum total of the activities and interactions of the cell units.

Thus grew the conception of the cell as the unit of life.

Eventually the cell theory was extended and applied to Pathology by Virchow, and to Embryology by Kolliker. While Brown discovered the nucleus, Wagner discovered the nucleolus. Others like Dujaedin, Schultz, Purkinje concentrated on the protoplasm. Once the general structure of the cell was established there was rapid progress. Cell division in animals was discovered by Flemming and in the plants the cell division was discovered by Strasburger. Chromosomes and their importance in mitosis was demonstrated by Waldeyer (1890). Fertilisation process was described by O. Hertwig (1875). Cytoplasmic inclusions such as cell centre, mitochondria and Golgi were quickly discovered and described. Thus CYTOLOGY, a separate branch of biology came into being.

Our knowledge in this science has advanced mostly due to the advancement and perfection of microscope and microscopic techniques. Though the microscope was discovered by Leuwenhock in the 17th century its capabilities were realized only in the 18th century. The utlimate aim of microscopy is to enlarge the image and to attain an increased resolving power. Fixation and staining techniques have advanced side by side. Phase contrast microscpoe, discovered by Zernicke, a nobel prize winner in 1953, has since attained perfection in contrasting a difference in intensity or colour between object and background, in other words, the structures produce alterations in the phase of the transmitted

radiation. The Interference microscopy is used in determination of weight, the polarization microscopy is used to study the mole cular orientation of cell structure of non living material, the ultra violet microscopy is used in histo-chemical studies and the flourescent microscopy is used in Virology (Table I & II).

TABLE—I

Method	Dimensions of Structures that can be seen	Structures that can be seen
1. Unaided eye and simple lens	0. 1 mm (100 & larger,	Organs
2. Light micros-copy	100 to 10μ	Tissues
3. X-ray micros-copy	10 to .2 (2000 Å)	Cells, bacteria
4. polarisation & electron microscopy	2000 Å to. 2 Å	Cellular structures
5. X-ray diffraction	Smaller than 10 Å	Arrangement of atoms.

TABLE—II

$$1 \text{ micron } (\mu) = \frac{1}{1000} \text{ mm or } 10{,}000\,\text{Å}, \text{ or } 1000\,\text{m}\mu$$

$$1 \text{ millimicron } (\text{m}\mu) = \frac{1}{10{,}00{,}000} \text{ mm or } \frac{1}{1{,}000}\,\mu \text{ or } 10\,\text{Å}$$

$$1 \text{ Angstrem } (\text{Å}) = \frac{1}{1{,}00{,}00{,}000} \text{ mm or } \frac{1}{10{,}000}\,\mu \text{ or } \frac{1}{10}\,\text{m}\mu$$

The Electron Microscope was introduced in 1940 and has contributed greatly to our knowledge of the cell structure. It is relevant here to point out some of the differences between the light microscopes ordinarily used and the electron microscope. The electron microscope uses instead of light, a beam of electrons with a wavelength of less than one Angstrom (A° -0.1m). Instead of

glass, it uses electromagnetic fields as lenses and condensers capable of refracting the beam of electrons, in the same manner as the light microscope handles light. Instead of the retina of the eye. the electron microscope produces the image on a floures-cent screen or a photographic plate. In this microscope, though theoretically the resolving power is less than 1Å engineering and technical problems are great and so far resolution of 6 to 8Å has been achievedi nfrequently. Resolution of 20 to 40Å is common. The main drawbacks of the Electron microscope is that specimens must be 'dry and ultra thin'. We therefore see under the microscope only structures that are no longer alive.

During the last few years new cytological techniques have been developed which have also contributed greatly to our understanding of the complex anatomy of the cell. These techniques include (a) cytochemical and histochemical techniques by the methods of differential staining and detection of specific substances (b) techniques of isolation by homogenization centrifugation and microsurgery (c) autoradiography and (d) tissue culture methods.

Principal types of living system

The distinction and recognition of the living world into plants and animals dates from the most primitive periods. With the advent of microscopy and the examination of the microbial world, the list was expanded to include a third kingdom called Prostista which include Algae, Protozoa, Fungi and Bacteria.

Taking Prostista into consideration, the living cells can be distinguished into two different kinds.

1. **Eucaryotic cells:** The cells occur in all plants and animals and in the more highly evolved members of Prostista, viz. Fungi, Protozoa and most Algae. The eucaryotic cell is characterised by the presence of a nuclear membrane and by the characteristic changes in the appearance of the nucleus during cell division.

2. **Procaryotic Cells:** These occur only in bacteria and in certain forms of algae. The nuclear membrane is absent and the process of cell division is not accompanied by the apperance of the distinct nuclear bodies characteristic of eucaryotic cells.

It is important to realise that the simliarities of the two type of cells out number their differences. Both sustain similar metabolic processes. Both are confined by celluar membranes. Both are endowed with genetic continuity and obey basiclly similar genetic principles.

Size of the cells

Cells vary in size and shape. The smallest living cells are found among bacteria 250m μ in dia. (1000 microns $[\mu]$ = 1 mm; 1000 millimicrons $[m\mu]$ 1 μ). It would seem that 200 to 250 mμ in diameter is the lower limit for the size of an active, living cell. The majority of cells lie the range of 0.5 to 20μ in diameter. There are also some truly giant cells which are highly specialised.

General structure and function of the cell

Though the cells may vary superficially according to different functions, most cells exhibit certain fundamental structures and function.

1. A living cell is bounded by a membrane which is usually about 100Å thick (1 Angstrom $= 10^{-8}$ cm.) This membrane is always characterised by definite selectivity as to penetration by foreign substances, permitting passage to some and acting as impassable barrier to others.

2. Division of the cell into nucleus and cytoplasm.

3. Cytoplasmic inclusions such as mitochondria, lysosomes, centrosomes, ribosomes etc.,

4. Membrane systems of cytoplasm called endoplasmic reticulam and Golgi apparatus.

5. In higher organisms the nucleus is separated from the cytoplasm by the nuclear membrane. The nucleus contains the important chromatin filaments of deoxyribonucleic acid (DNA). These filaments appear as distinct chromosomes during cell division. The functional common features exhibited by the cells are:

1. They utilise energy from outside sources to organize atoms and molecules from the external environment and synthesize macromolecules typical of their own structure.

2. They perpetuate information for their own synthesis through repeated cycles of information.

3. They control their internal environment in such a way as to create the most suitable conditions for their metabolism.

4. They regulate their component reactions so that these work in harmony.

Cellular differentiation

In single celled animals, all the functions of life are carried out in one cell. In multicellular organisms, groups of cells are specialized in their functions. Such specialised cells are said to be differentiated. In general, the cells that are present early in the development of an individual are relatively undifferentiated.

The circumstances which give rise to cyto–differentiation are little understood. The nucleus is generaly assumed to be the same in all generations of cells. The constitutents of cytoplasm may be altered and they are followed by changes in the cytoplasmic chemical processes resulting in functional and anatomical differentiation of cells.

Specialization of function of cells is generally accompanied by reltive loss of other functions. For example, many cells do not multiply following differentiation as in nerve cells-which after a relatively young stage do not reproduce but may continue to grow by increasing in size. Some types of cells however, continue to grow even in the older individual, e.g. certain cells of the skins, of the reproductive system etc. In continuously growing tissue, of this kind, the finally differentiated cells do not however reproduce. Thus it may not be always possible to demonstrate in any particular cell, all types of cellular functions that living things are supposed to show.

Occasionally, cells regain the lost ability to reproduce. They lose their differentiated conditions and begin to grow and reproduce in an unorganized manner. The various cancers are examples of this condition.

Differentiation of tissues

The fertilized egg, called a **zygote,** *is* really a one-celled organism, and from it develops a complete animal with all its structures and functions. The major process which makes it is **differentitation** by which the various cells become

unlike. Tissues are differentiated by **histogenesis.** Five major tissues are differentiated: (1) epithelial tissue from all three germ layers (2) connective or supporting tissue, from mesoderm (3) muscle tissue from mesoderm; (4) nervous tissue from ectoderm; and (5) vascular tissue from mesoderm. A **tissue** is a group of similar cells (together with associated cell products) specialized for performance of a common function. The study of tissues is **Histology.**

CHAPTER XVIII

The Functional Anatomy of the Cell

Cells constitute a remarkably varied group of organisms. Nevertheless, almost all of them have certain structural and chemical properties in common. It is therefore convenient to think of a 'typical cell', as a cross between an animal and a plant cell, with no highly specialized functions but with a capacity to differentiate in many ways. This convention is useful and convenient to study.

In the most general terms the following structural elements recur in all cells;

1. All cells are surrounded and confined by a thin film called a **membrane.**

2. A basic division of the cell contents exists between an interior **nucleus** and the surrounding **cytoplasm.**

3. In the cytoplasm, a number of granules exist-among the most important are the **chloroplasts** of green plant cells and the **mitochondria.** Other organized elements of the cytoplasm are **lysosomes & centrosomes.** In addition the cytoplasm appears to possess a system of internal membranes called the **endoplasmic reticulum.** A large number of small granules appear on its surface, called **ribosomes.** The **Golgi apparatus** is also closely associated with the endoplasmic reticulum.

4. In the higher organisms, the nucleus is separated from the cytoplasm by the **nuclear membrane.** The nucleus contains the **chromatin** filaments, which contain DNA and which are rather indistinct in the interval between cell divisions, but appear as **chromosomes** during cell division. Another prominent body on the nucleus is the **nucleolus,** which undergoes cyclic changes in appearance during cell division. It is rich in RNA and may be the site of protein and RNA synthesis.

The ultra structure of the cell

A great revolution has been brought about in Cell Biology by the discovery of the electron microscope, which has revealed to us

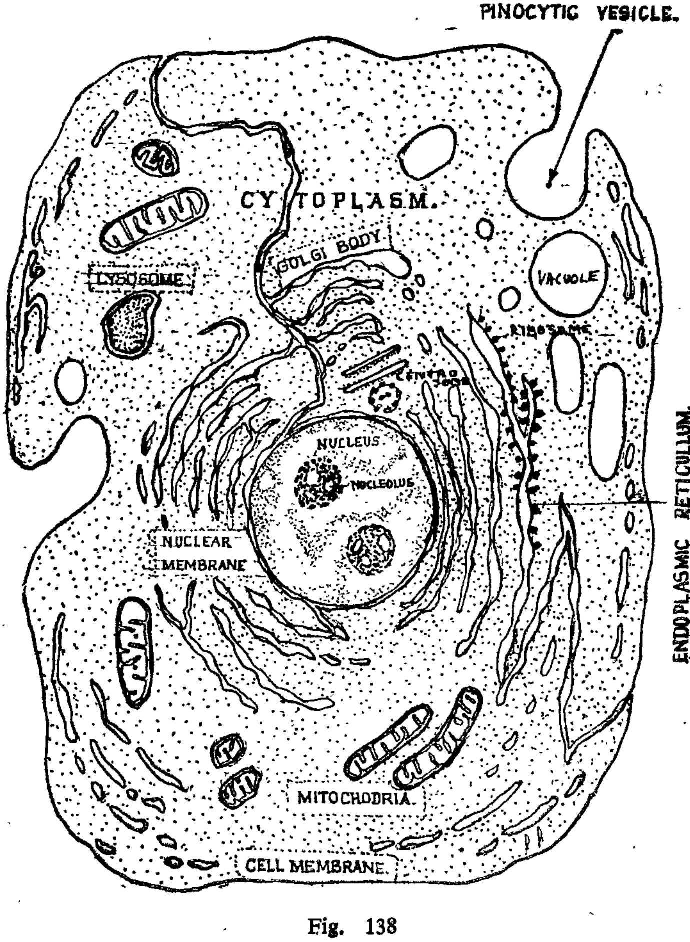


Fig. 138
Cell Ultra structure

structures which could not be ordinarily seen by the light microscope. Other techniques used in the study of the cell have given us an insight into the chemical structure of the organelles and have made it possible for us to understand to some extent the complexities in the functioning of the cell.

I. THE PLASMA MEMBRANE

The cell surface is delimited by a definite, osmotically active plasma membrane, or plasmalemma, which is capable of lowering the rate of penetration of molecules as well as discriminating between them. In plant and protisitan cells, the plasma membrane is covered with a coat, the cell wall. In many animal cells this is not easily seen and its existence is a matter of dispute. The properties of the cell membane may be profoundly modified by this coat and its chemical structure is usually distinctive of particular cells or tissues.

Structure of the cell membrane: Although precise details are still in dispute, it is generally agreed that the typical cell membrane is about 75Å thick and the basic structure is almost certainly a double layer of phospholipid with the non-polar groups apposed and the polar groups turned outwards. Attached to the lipid layers are probably protein layers both on the outside and inside. This structure is called the 'unit membrane' by Robertson. Until recently, it was thought to be characteristic of all plasma membranes and the delimiting membranes of subcellular structures. But, while the origin and basic structure of all membranes may be similar, distinct structural and chemical differences have evolved which may confer organ and tissue specificity. So the unit membrane hypothesis has been criticised in this context and a 'sub unit' concept has been suggested. According to this hypothesis, most membranes may consist of repeating subunits built of structural protein associated with phospholipids and other compounds. Each membrane would possess its own specific structural unit. Thus, newer techniques of microscopy such as negative staining technique, electron microscope and X-ray diffaction analysis have revealed that the original Danielli-Davson model must be revised. The main structural element of most membranes may not be the lipid as once thought, but protein. There is no question however that the variation and complexity of the phospholipid component of membranes have direct bearing on the specific function.

To explain free entry of very small particles, it is proposed that the lipid layer is penetrated at intervals by **pores** with a dia meter of about 7-10Å — These pores are probably lined by positively charged groups since cations are specifically excluded.

The plasma membrane is not tightly stretched over the cell but usually the surface folds inward or outward showing different kinds of modifications in different types. These are (1) **microvilli**-minute folds into the cytoplasm (2) **interdigitations**—fold like structures between the cells and (3) **desmosomes**—thickened regions of plasma membranes with inner filaments.

Properties of the cell membrane

(a) **Permeability**: The ease with which substances pass through the cell membrane depends on three factors: (1) The greater the solubility of a substance in lipid solvents, the more readily it passes through the cell membrane. (2) Certain very small ions, with ionic radii of less than 7Å can pass readily through the cell membrane (3) Substances such as carbohydrates, proteins and sodium do not fall under any of the above categories. There is evidence that for such substances there are special mechanisms to facilitate their passage through the cell membrane.

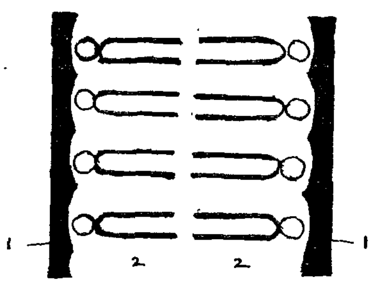


Fig. 139
Cell membrance (a model)
1. Protein layer
2. Fat or lipid layers

(b) **Surface tension** is very low and this is strong evidence for the absorption of protein to the outisde of the lipoid layer.

(c) **Electrical properties**: The cell membrane exhibits high electrical resistance and this fact proves that it consists largely of lipoid material.

Chemical properties: The chemical complexity of the cell membrane is intimately linked with the physiological and biochemical process of the entire cell. Particularly characteristic of cell membranes is the structurally orientated **ATPase system** associated with active transport mechanisms. The **gangliosides** are another class of specifically oriented compounds present in abundance in brain and liver and probably characteristic of most cell membranes.

Functions of the cell membranes

1. **Transport mechanisms:** The protection which is provided to the cell to maintain its internal environment depends entirely on the plasma membrane which controls selectively the substance that enter or leave the cells.

To understand the passage of materials in and out of the cell is one of the greatest problems in biology. Much experimentation has been done and many important facts have been discovered though all the problems are not solved yet. It would be relevant therefore to consider some simple facts about the movement of molecules and their behaviour when confronted by a membrane.

Movement of molecules

All molecules, whether in gas, liquid or solid state are in continuous motion. They move in a straight line until they meet another; then they bounce off and take a different direction, producing a zigzag path (Brownian movement). The total molecular activity of a substance in a particular region results in a **diffusion pressure.** When two groups of molecules of different kinds are placed in contact, a mixture of molecules gradually takes place until eventually, they are thoroughly mixed. This phenomenon, called diffusion may therefore be defined at the movement of molecules or ions from a region of greates dygusim pressure to region lesser diffusion pressure. This phenomenon is fastest in gases and slowest in solids. It can even occur if a membrane separates the two groups of molecules, **provided** the membrane is fully **permeable,** and will allow both types of molecules to pass.

When the membrane allows only one type of molecule to pass through it is called **semipermeable**-then a different situation results. One type of molecule is not permitted to pass trough the semipermeable membrane, whereas, another can pass readily in **either direction.** Thus after a period of time on one side of the membrane only the rebuffed molecules will be found, whereas the favoured molecules will be found on both sides of the membrane. Therefore more materials will be found on one side of the membrane than on the other.

When the two groups of molecules, separated by the semipermeable membrane are placed in a container, open at the top, the height of the column will be different on the two sides of the membrane. If, in addition a piston is inserted above the column which contains the molecules that do not diffuse through the membrane, it can be observed that the molecules entering from the opposite column accumulate and the piston is raised gradually. There is therefore a definite pressure exerted againt the piston which can be measured in a crude manner by placing weights on the piston until it rises. This pressure which is the pressure necessary to prevent the rise of the piston is called osmotic pressure. In osmosis as well as diffusion, the movement of molecules is from greater to lesser concentration. When the concentration of molecules is uniform throughout the mixture, the number of molecules that move in opposite directions is equalized and an **osmotic balance** is said to have developed. Osmosis therefore is the movement of molecules from greater to lesser concentrations through a semipermeable membrane.

Factors which control osmosis

a. **Degree of viscosity:** The presence of potassium or calcium influences the movement of molecules in the plasma membrane, because potassium renders the membrane more fluid (sol state) and calcium makes it more viscous (gel state).

b. **Size of molecules and pore size:** The size of molecules bombarding the surface of the protoplasm influences the entry of molecules. To explain this, the pore concept has been put forward.

c. **Ability of the fatty layer to dissolve related substances:** The ability of fats to dissolve related substances affords a partial explanation for anaesthesia. Chloroform and ether dissolve rapidly in the fatty substances on the plasma membrane at the expense of and to the exclusion of other substances. A blanketing of the surface results in a lowered oxygen intake followed by a lowering of metabolic activity of the protoplasm (Brain cells are especially high in fat content).

d. **Electrical charge carried by the plasma membrane:** Protein molecules have the property of being either positively charged or

negatively charged and since the proteins are such an important part of the membrane, the surface of the protoplasm may have either a positive charge or negative charge. Four chemical particles which are important to the protoplasm and which are normal constituents of the environment are the positively charged ions, sodium ($Na+$) and potassium ($k+$) particles and the negatively charged ions, chlorine (Cl^-) and hydroxide (OH^-) particles. A membrane which is positively charged will repel the Na and K ions but will attract negatively charged Cl and OH and vice versa.

Whereas the movement of molecules in osmosis is determined by the concentration gradient between the two surfaces of the membrane, in Active transport the direction of movement may be against the apparent concentration gradient; but the most essential feature is that a steady supply of energy is necessary to maintain those features of the surface on which active transport depends.

During the last twenty years, the mechanisms involved in active transport have been extensively studied and there is increasing evidence of enzymatic activities at the cell surface.

1. Thus, one of the main **functions of the plasma membrane** is to restrict the diffusion of molecules and ions. There are three general mechanisms whereby it is carried out; viz, passive diffusion, exchange diffusion and active transport. The actual mechanisms by which larger molecules gain entry into the cell is not understood. There is conflicting evidence that energy may be required but it seems quite likely that in the presence of suitable osmotic gradient, glucose can enter or leave freely without utilizing energy. By selective exclusion of certain molecules and by active pumping of substance in or out of the cell, the cell membrane maintains the internal environment. Solid particles may be ingested through the plasma membrane by **phagocytosis.** It can be typically illustrated by the way an amoeba ingests its prey. Liquid substances may be incorporated into the cell by the plasma membrane by **pinocytosis.** It is believed that macromolecules are absorbed into the cell from the environment in this manner.

2. **Conductivity:** This is another important property of the cell membrane. When the cell membranes are depolarized by

minor stimuli, a wave of excition may travel over its surface. This behaviour, observed in many kinds of cells is the basis of the excitability of the nerve cells.

3. Specialized cell membranes such as myelin sheaths which surround the nerve cells, provide an effective insultation to prevent leakage of charge from axons.

II. CYTOPLASM (Ground substance)

The interior of the cell is rich in a mixture of macromolecules, smaller organic compounds and ions. These comprise the ground substance. The colloidal material has the usual property of having a viscous flow like the fluid and elastic deformation like a solid.

There has been much discussion for many years over the existence of an organized framework or cytoplasmic skeleton in the ground substance. No such cytoplasmic skeleton is visible under the electron microscope however. Mobile extensions of cytoplasm as flagella and cilia and the fibrillar organization of centrioles show that the cytoplasmic matrix is capable of a number of forms of fibrillar organization that seem to be involved in the conversion of chemical energy into mechanical work.

In the cytoplasmic matrix suspend many organelles each of which is surrounded by a membrane. These are the mitochondria, the chloroplasts (in green plans) the lysosomes, the nucleus and various other granules such as fats etc.

III. ENDOPLASMIC RETICULUM

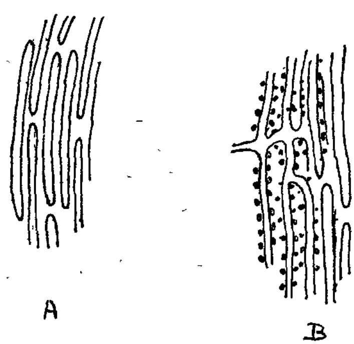


Fig. 140 Endoplasmic reticulum
A. Smooth B. Rough

If the plasma membrane is examined carefully, it will be seen that some of the invaginations from the plasma membrane lead right into the depth of the cell. These channels connect with a complicated set of vesicles which interlace the structural matrix of the cell. These membrane systems are called the endoplasmic reticulum.

Three structures constitute the endoplasmic reticulum.

a. **Smooth endoplasmic reticulum:** These are intracytoplasmic membranes consisting of filaments or lamellae, 50Å thick and parallelly arranged.

b. **The space enclosed within** the membranes presenting a tubular or canalicular appearance.

c. **Small dense opaque granules,** 130 to 150Å in diameter, of RNA-protein may occur attached to the endoplasmic reticulum, forming the rough or granular endoplasmic reticulum.

Occurence : The reticular elements of the cytoplasm are extremely prominent in some cells such as the exocrine cells salivary gland cells etc. Tubular cells of the kidney and muscle cells have less. They are absent in red blood corpuscles and some embryonic cells. Smooth membranes are found in certain differentiated cells particularly those concerned with the formation of steroids.

Dimension : The dimensions of the endoplasmic reticulum are similar to the plasma membrane. The membrane is often directly continuous with the outer parts of the nuclear membrane. It is thought that it may originate from the outer part of the nuclear membrane.

Function:

1. The endoplasmic reticulum is intimately involved in protein synthesis: electron transport and ion transport steroid and fat metabolism and various oxidative reactions.

2. The canalicular appearance of this organelle suggests a feeding of the products of these reactions to other parts of the cell. Thus it transports the metabolic products to other parts of the cell.

3. It is a source of origin of some other membranes.

Microsomes: Claude (1943) discovered a number of minute submicroscopic bodies which could only be sedimented with very high sedimental forces. These are not naturally occuring structures but are artifacts of the isolation procedures. When examined under the electron microscope, they appear to be 500 to 3000Å in length

with large amount of RNA. They are tubular and vesicular fragments of the endoplasmic reticulum, mostly from the rough type.

IV. RIBOSOMES

These can be separated and isolated from the rough reticulum. They have been described by Palade.

Structure: The structure can be observed directly through the electron microscope. Another method used to study its morphology is called analytical ultra centrifugation. The substances are subjected to very high ultracentrifugal forces and speed (e.g. 100,00 xg for 1 hour) in a medium of suitable ionic strength, pH and Mg^{++} concentration. With this technique, H.K. Schascman, J.D. Watson, A. Tissieres and other have shown that the ribosome is made up of two unequal particles. The particles are named with reference to the Unit of Sedimentation Velocity (S). In E. Coli, for example, one of the particles sediments at the rate of 30S and the other at 50S. The structure of the ribosome in E. Coli as given above is markedly effected by the concentration of Mg in the solution medium. In the cell, the two particles are normally seen to be joined together in a single entity which sediments at 70S (the reason is that the two S values are not additive) because the shape of the particle influences its rate of sedimentation). It is believed that 70S particle is the functional biological unit within the cell of E. Coli. Patersmann and Pavloec (1966) have isolated ribosomes from mammalian cells. They sediment at approximately 80S and are composed of two subunits of 33S and 54S.

Chemical composition: The ribosomes are composed mostly of proteins and RNA. Other substances found are Mg. and, or polyamines. Warner (1966) has distinguished three classes of proteins designated as a, b and c. The molecular weights of these proteins vary from 12,000 to 25,000. Investigations of Palade, Seikevitz and Zamecnik have clearly established the nature and importance of these particles. The RNA comprises 85% of the total RNA of the cell. It has been established by auto radiographic studies that the RNA is synthesized in the nucleus, and is catalysed by RNA polymerase and is dependent on DNA. The ribosomal RNA has a small number of methylated bases. Ribosomal RNA and protein have a relatively long life (5 days).

Function : The ribosomes serve as a work bench for the synthesis of protein in the cell. They are present in almost all tissues, but there is a definite correlation between the concentration of particles in a tissue and the capacity of that tissue to synthesize proteins.

V. GOLGI APPARATUS

The Italian microscopist Camimo Golgi, in 1898, discovered certain previously unknown bodies in the cytoplasm of nerve cells in the barn owl by using a special silver stain. Santiago Ramon Y Cajal, an eminent Spanish histologist had also reported several years earlier the same structures. For their studies, which were conducted independently Golgi and Cajal were jointly awarded the Nobel Prize for physiology and medicine in 1906. Since then numerous workers have investigated the Golgi apparatus and various terms such as dictyosomes, Golgi bodies, lipochondria, internal reticulum apparatus, canalicular system, trophospongium etc. have been given to this complex structure. Although the Golgi apparatus was given much attention and numerous papers were published on the subject, there were doubts about its existence and were considered as artefacts by some till the 1950s, when Dalton removed all doubts regarding its existence by his electron microscope studies. He demonstrated that the Golgi apparatus exist in all cells and that it consists of one or more stacks of tiny flattened sacs or saccules.



Fig. 141 Golgi bodies
1. Flattened sacs
2. Vesicles.

Structure: The structure of the Golgi apparatus varies in form, size and location within the cell; but its organization is basically constant. In the exorcine cells of mouse pancreas, Sjostrand and Hansen recognised the following elements:

1. **The Golgi ground substance:** It is a homogenous substance granulated or reticulated.

2. **The Golgi membranes:** A system of flattened sacs which can be readily seen in theordinary histological preparations. These sacs are called cisternae or lamellae and are composed of double. membranes of lipoid nature, in the exocrine cells of the pancreas

The lamellae are 60-70Å in thickness and the interspace is about 70Å. There are no particles attached to the membranes.

3. **Vacuoles:** There are Globular and irregular in structure and formed mainly by the enlargement and swelling of the sacs.

4. **The Golgi granules:** These are clusters of small vesicles ranging in size from 40 Å to 600 Å. Present near the periphery of the complex. They are considered to be derived from flattened sacs by constrictions and subsequent separation from them. The most consistent and fundamental component of the Golgi is the cisternae which are the importantaspects of the Golgi apparatus.

Location: The location of the Golgi within the cell shows variations, depending on cell types. Generally, it is found above the nucleus and partially enclose the centrosome. In some cells, it is distributed throughout the cytoplasm. In secretory cells, it is found between the nucleus and the secretory poles of the cell.

Physical properties: Its constituency is generally fluid but may vary depending on the nature of the cell. Specific gravity is less than that of the ground cytoplasm.

Chemical composition: It is complex and variable and is not yet fully understood. The fact that it is usually soluble in fat solvents suggests that it may contain fatty or lipid material. Analysis of the Golgi material isolated from intestinal cells of rabbit by density gradient ultracentrifugation shows the presence of carbohydrate protein complex. Bourne has reported the presence of Vitamin C. It is however not wise to to be dogmatic about the localisation of this material. There are no nucleic acids. Golgi is poor in mineral content. Berlin (1967) has demonstrated recently the presence of mucopolysaccharides inside the Golgi complex, of goblet cells of the intestine. Some enzymes such as alkaline phosphatase, acid phosphatase, nucleoside diphosphatase and thiamine pyrophosphatase are present. The relationship of these enzymes to the process of segregation, concentration and secretion in Golgi is not known.

Origin : There are three different views as to how the Golgi originate in the cell (1) That they arise denovo. (2) That they

arise from pre-existing material by deviation or fragmentation. (3) That they arise directly from one of the cytoplasmic membranes of the cell.

Function: The activity of the Golgi apparatus and its function in the cell have been matters of controversy in spite of the vast amount of literature on the subject. Recent investigations throw more light on this aspect and the data available indicate that the Golgi is one of the main chemical factories of the cell, producing substances of great importance in all living organisms.

1. The Golgi may function in various secretory pyenomena as a segregation and concentration area. Electron microscopic studies and autoradiographic studies suggest that in the formation of glucoprotein secretions such as enzyme and mucous, the protein synthesized perhaps in the rough endoplasmic reticulum is channelled through the Golgi where carbohydrate is added to the protein to form glycoproteins. Recent tracer experiments have shown that Golgi apparatus is also the site where sulphate is added to the carbohydrate part of the glucoprotein.

2. Tracer techniques have also shown that in the cartilage cells, Golgi region secretes mucopoly saccharides.

3. In some cells, the Golgi gives rise to lysosomes.

4. Bowen's brilliant work of 1929 and later by Faucett demonstrate a definite relationship between the Golgi and the developing acrosome of spermatids.

Examination of principal cells of small intestine of mouse after a 24 hour fast and 40 minutes after feeding them with corn oil and after its absorption shows that the absorbed lipid is stored in the Golgi.

5. Cohen has shown that the golgi may be involved in the synthesis of phospholipids.

6. Birbeck and Dalton have shown that Golgi represents the site of melanin granules in several mammalian tumour and cancer cells.

To summarise, the Golgi is the most controversial of all the cell organoids. It seems to be a creative mechanism in the cell and the main agency for building a variety of large carbohydrates that serve many vital purposes.

VI. MITOCHONDRIA

Mitochondria (chondriosomes) are membranal structures which are centres of the cell's respiratory metabolism where the foods of the cell are oxidized to CO_2 and H_2O and the energy released is used to convert ADP to ATP. Perfection of electron microscopy has produced tremendous advances in our understanding of the structure and function of the mitochondria. From these extensive studies of cytologists and biochemists, a complete picture has evolved.

Electron microscope studies of mitochondria : The mitochondrion is a structure bounded by a limiting double membrane and found in all cells except the bacteria and mature red blood cells. The outer membrane, about 140 Å thick, double layered in section, with two electron dense lays each 35Å in thickness, is stretched tightly round the organelle. The two layers in the outer double membrane are separated by a less opaque space. The interior of the mitochondrion contains double membranes or plates, for the most part oriented at right angles to the long axis of the mitochondrion. The **matrix** is generally homogenous and contains small granules of high density and finely filamentous material which is probably DNA. The matrix appears semisolid in character and lipoprotein in nature.

The plates or cristae which are found in the interior of the mitochondrion, occasionally extend right across the intramitochondrial space. They are 170Å thick and the individual electron dense layers are 40Å thick. The relations of these layers of the plates to those of the bounding double membrane are curious. Palade regarded the plates as folds of the surface membrane and called them cristae mitiochondrialis. However, it has now been demonstrated that with a few exceptions, the space of the plate is never continuous with the space of the membrane; generally the two dense layers of the plates meet at the edges,

so that they enclose a space, but sometimes the two layers make separate contact with the inner layer of the enveloping membrane. Moreoever, not all mitochondria are partitioned by the cristae. Those of certain ciliates contain twisted mass of closely compacted tubules. The number of cristae directly affects the capacity of mitochondria. The greater and larger the cristae, the stronger the capacity to carry on oxidative reactions. In some protozoans, the inner surface of the mitochondrial membrane is associated with small granules.

Each mitochondrial inner membrane is quite complex. While the structures may correspond to the unit membrane hypothesis of Robertson, recent evidence suggests globular micellar arrangement, with projecting subunits. Each subunit consists of a base, a stalk and a knob like head.

Chemical Composition: The mitochondrion is a highly organized though not rigid, solid. Kolliker's analysis of liver mitochondria has shown that lipids account for 25% to 30% of the dry weight and 2/3 of this is phospholipid. The proportion of protein amounts to between 65 to 70% of the dry weight. RNA content is low, amounting to a few percent. In recent years, Nass and Nass (1963) have demonstrated the presence in the matrix, of fibre like material, which appears to possess the characteristic of DNA. It appears that it may be circular viral type. Several attractive possibilities are there for this extra nuclear mitochondria located DNA. May be the mitochondria have their own protein synthesizing machinery, or may be, the DNA functions as a regulatory system, or perhaps, it is responsible or duplication of the organelle, or may be it is nongenetic in function. Respiratory enzymes such as cytochrome oxidase, reductase, transaminase, coenzymes, a octanooxidase, fatty acid oxidase etc. are also present.

Enzymic properties and functions of mitochondria : One of the most important general conclusion to be drawn from the studies of mitochondrial preparations is that the mitochondria is an organized functional unit. An elementary knowledge of the biological oxidations in the cell is essential for an understanding of the functions of the mitochondria.

The cell is the seat of biological oxidations which furnish energy for the various activities of the cell. This energy comes

from the food materials, chiefly glucose, which all animals must consume in order to live. The process by which glucose is broken down and the energy locked in it is released for use in growth, manufacture of new protoplasm, synthesis of many compounds secretion, contraction of muscle etc. is very complex, and can be divided into two major groups of steps.

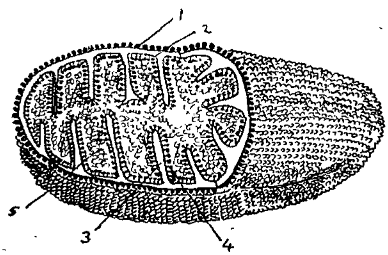


Fig. 142

Mitochondria

1. Outer membrane 2. Inner membrane 3. Cristae 4. Electron transport particles 5. Matrix

1. Anaerobic step takes place in the absence of oxygen and is called **glycolysis.** It is not such a simple process as it sounds, however. The glucose carried by the blood to the cell combines with a phosphate group to form glucose-phosphate. This process is called **phosphorylation** and is controlled by a special enzyme.

Next, this glucose phosphate, by a series of steps, each of which is catalysed by an enzyme is finally convereted into **pyruvic acid.** During these reactions, energy is released at certain steps, which are trapped in the energy-rich bonds of ATP (adenosine tri phosphate) in which form the energy is available to the cell for use. These anareobic reactions take place in the cytoplasm of the cell. The pyruvic acid is further oxidized to yield carbondioxide and water if oxygen is available; if not, it is converted to alcohol, acetic acid etc.,

2. The second step of energy yielding reactions involving oxygen is called by various names, such as **Kreb's cycle,** citric acid cycle, and tricarboxylic cycle. It is **aerobic** and takes place in the mitochondria. This consists of a series of reactions by which citric acid is oxidized to carbondioxide and water. The energy that is released by these reactions, is much more than the energy yielded by glycolysis. The reactions may be summarized as follows:

a. The pyruvic acid is first converted to **acetic acid** which reacts with **coenzyme A** (from the vitamine panto thenic acid) to form **acetyl coenzyme A.**

b. Acetyl conenzyme A then unites with **oxaloacetic** acid to form **citric acid.**

c. In a series of cyclic steps, each controlled by a special enzyme, the citric acid is broken down through eight different organic acids back to oxaloacetic acid which then continues the cycle again. In third cycle, carbondioxide and hydrogen are given off by special enzymes, and the hydrogen atoms are transferred by **flavoproteins and cytochrome enzyme** system to oxygen which unites with the hydrogen ions to form water. This process is called **cellular respiration.**

Two points are noteworthy.

1. Since the last step in the Kreb's cycle leads to the formation of citrate, the sequence of reactions is cyclic and will continue as long as oxaloacetate continues to be formed.

2. Any member of the cycle can be converted into CO_2 and H_2O and if supplied swith any one of these acids as substrate, the mitochondria will bring about its complete oxidation.

The Kreb's cycle is also used in the oxidation of fatty acids and aminoacids of the other two classes of foodstuffs and is the chief source of energy in the cell. The main significant aspects involved here are, the transfer of electrons and the mechanism for coupling the transfer of electrons to the phosphroylation of ADP and ATP. The cristae of the mitochondrion are directly related to these oxidative activities.

Functions attributed to the outer membrane : The various enzyme systems involved in the biological oxidations (aerobic) do not lie free within the mitochondrion but form an association with the lipid membrane on which they are arranged.

1. The enzymes and cofactors invloved in the Kreb's cycle are said to be located in the outer membrane of the mitochondria.

2. The outer membrane is also said to contain **monamine oxidase,** which is apparently localized exclusively in the mitochondrion. In addition to functioning in the metabolism of catecholamines, it may also be involved in control of respiratory

activity, and as a catalyst in the deamination of tyromine and may also exert a regulatcry effect on theoxidation of succinate.

3. The outer membrane may also contain a number of enzymes not associated with the Krebs cycle or oxidative phosphorylation. These are associated with the synthesis of material necessary for the mitochondrial activity such as phospholipids, fatty acids, phosphoproteins and possibly cytochromes.

Functions attributed to the inner membranes: The electron transport chain and reactions involved in the oxidative phosphorylation are located in the inner membranes. The fragments obtained by subfractionation techniques of sonic vibration etc., and similar procedures have demonstrated that the mitochondria consists of a large number of orderly, recurring structural units, each of which contains an array of respiratory carriers in a specific ratio. These fragments called **electron transport particles** (ETP) reveal relatively intact assemblies of enzymes capable of electron transport and coupled phosphorylation, but do not show any substrate level enzymic apparatus or Kreb's cycle intermediates. With the modern techniques, the ETP has been further fragmented into 'elementary particle' (EP) which are 80 to 100Å in diameter. These are considered to be the basic structural subunits of mitochondria. The EP consists of a head piece and a stalk by which it is attached to the inner membrane. Recent studies indicate that the stalk is concerned with electron transport and the head piece may represent the terminal step in oxidative phosphorylation, that is, formation of ATP.

It must be emphasised here that the data on mitochondrial function are still in the fcrmative stages and prccedures are being constantly improved and biochemical data are interpreted.

Occurance: Mitochondria are not confined to the Metazoa and Metaphyta. In the Volvocina they have been identified as continuous superficial network in fixd materials, but in other orders, the chondriosome may consist of discrete threads or rodlets as in the cells of Metazca. Mitochondria are abundant in Sarocodina and in the Ciliata and indeed seem to be present is all Eucaryotic cells.

Shape, Movement and behaviour in the living cell : Mitochondria may occur as spherical or rod shaped particles or as filaments; they may change in shape in the cell according to phases of activity or of nutrition. Even in the same organism, they may differ in shape in the different tissues. In the living cell the filaments show no constancy in the diameter, and they may appear beaded or branched. During cell division, they undergo profound changes in step with nuclear division. Though there is no evidence of regular multiplication of mitochondria the occurence of transverse fission is a common event in the rod shaped and filamentous forms. Under dark ground illumination and phase contrast, the mitochondria of tissue culture cells are found to be in constant movement both by moving within the cell and by flexion of individual threads.

Origin: There are three opinions regarding the origin of the mitochondrion in the cell (1) That they develop from the accumulation of microbodies into the cytoplasm. (2) That they arise denovo. (3) That they arise from the plasma membrane.

VII. LYSOSOMES

These are particles about the size of mitochondria which are rich in digestive enzymes. They vary in structure but are always surrounded by a lipoprotein membrane. Sometimes this is single unit membrane, but often it consists of many layers of membrane which may be regularly coiled or folded. They are thought either to represent pinocytic vacuoles or to be special digestive vacuoles. In damaged or dying cells they undergo lysis and release digestive enzymes into the cytoplasm. Hence they may play a particularly important part in metamorphosis, embryonic morphogenesis and the response to injury.

VIII. CENTRIOLES

The centrioles or centrosomes are present in all animal cells capable of division. They become visible by ordinary microscopy only when the cell divides. Under the electron microscope the centriole appears as a cylinder 150 mμ in diameter, with an inner area of lesser density. Small rods or tubules of 150 to 200Å oriented parallel to the axis could be distin-

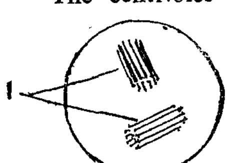


Fig. 143 Centrosome
1. Centrioles

guished. Each cylinder is made up of eleven fibres, with two in the centre and nine on the outside. This is the structure of cilia as well as flagella. Pericentriolar structures have been observed. They may appear as dense masses of about. 700Å sometimes attached to the wall of the centriole. Some interpret these structures as daughter centrioles. When there are two centrioles, they are disposed at right angles to one another. The position of the centriole is in general fixed for each type of cell. Often they are surrounded by the Golgi. The centrioles are made of RNA.

Function : The centrosome appears to initiate cell division and the centrioles are also involved in the formation of spindle and astral rays, which are formed by protein molecules by S-H bonds.

IX. NUCLEUS

The grounds on which Robert Brown (1833) discriminated the nucleus of the cell were due to its being slightly more opaque than the cell membrane. Yet, the notion that it is a prominent part of the cell organization appears already in the statement of the cell theory. What sustained the conception of the primary role of the nucleus was its visible participation in cell division and fertilization.

All animal and plant cells contain well defined nuclei, surrounded by a membrane. The number of the nuclei may vary. Bacteria contain a nuclear region, not bound by any membrnae. Whereas the cytoplasm seems to have many functions, the nucleus is mainly concerned with the preservation and exact reproduction of information for it is now established beyond all doubt that it contains genes located in the chromosomes. Studies of the cell after enucleation morotomy and other methods of studies with centrifugation and isolation techniques show that the nucleus plays a central role in the direction of cellular activity, differentiation of the cytoplasm and in the maintenance of the intra cellular RNA content.

Morphology: The nucleus is generally spherical, and variable in size. The size and shape are determined by the DNA content. The smallest nucleus is one micron or less as in the fungi and bacteria. The largest nucleus may be several hundred

microns in diameter. The average range of volume of the nucleus is 8 to $2000^3\mu$. The number is variable according to the functional demands of the cell, which may be met in either of the three ways: (1) Polyploidy-number of sets multiplied (2) Poly teny -number unchanged but the amount of chromatin (chromosomes) multiplied as in the salivary glands of some diptera (3) Multinuclearity-achieved by their either (a) multiplication of nuclei without division of the cytoplasm or (b) a syncytium of nuclei formed by the fusion of cells.

Structural components of the interphase nucleus

The nucleus of the cell which is not dividing is called the interphase nucleus. When observed in living cells by the light microscope, the intra nuclear structures are not clearly visible but under the phase contrast microscope, the chromonemata, chromocentres and nucleolus become visible. About twenty years ago, the fact that the chromonemata were not visible under the light microscope was disturbing and it was difficult to see how something resembling chromosomes could persist in an optically homogenous nucleus. But modern microscopy has revealed that these structures do persist and that they are invisible because of minute differences in refractive index.

The interphase nucleus is composed of the following parts:

1. Nuclear membrane (Karyotheca)
2. Nuclear sap (Karyolymph)
3. Chromatin
4. Nucleolus

1. Nuclear membrane: The nuclear membrane separates the two cellular phases of the cell, viz. the nucleus and cytoplasm, which are different morphologically and functionally. It is not generally visible under the light microscope but may be seen under the electron microscope and has been described by many, Callan and Toulin being the first to do so.

In structure, the nuclear membrane probably has the general properties similar to the cell membrane and like it, the structure is perhaps a unit membrane of trilaminar nature. The outer and the inner membrane each measure about 90Å with a space

of about 140Å between them. Ribonucleic protein granules may be found on the outer membrane. Pores, ranging from 500 to 1000 Å may interrupt the membranes. The pores may be transient and in some cells they appear as tubular prominences (annulus) extending from the cytoplasm to the nucleus. The nuclear membranes appear to be tough in the interphase cell for it remains intact in conditions which repture the cell membrane. Yet, at every cell division, it fragments into vesicles and sheets of flat membrane bound cavities which are indistinguishable from endoplasmic reticulum. Function of the membrane is mainly to transport materials in both directions.

2. **Nuclear sap:** It is the ground substance on which the chromatin lies. It is composed of proteins which are mainly nonbasic. There is no nucleic acid in the sap.

3. **Chromatin:** The interphase nucleus, after fixation presents a network like appearance with darkly stained granules, the chromatin. Evidence from observation of living cells in mitotic cycle at various levels of technical sophistication demonstrates that the integrity of the chromosome is maintained through interphase. Therefore it is reasonable to regard chromatin at interphase as a reduced, unfolded or extended chromosomes associated with additional materials which are probably metabolic. In the resting nucleus, the chromatin is diffusely distributed in the nucleus and coils up tightly into chromosomes at the time of cell division. The chemical organization of chromatin can be deduced from the biochemical analysis. The chemical composition seems to vary according to the metabolic activity of the cell. Generally, it is composed of DNA, histones and residual proteins.

The chromatin may be differentiated into **euchromatin** and **heterochromatin** on cytological basis, though their genetical distinction is vague. The euchromatin do not stain as much as heterochromatin, which stain very dark. It is said that the euchromatin region carry the genes while the heterochromatin region is said to be devoid of them. Further the heterochromatin remain condensed during interphase and are said to have a higher RNA content than the euchromatin.

4. Nucleolus: It was discovered by Fontana in 1781 and named by Bowman in 1840. It is visible under the light microscope as spherical body, homogenous, though small vacuoles may also be seen. Living cells observed with phase contrast microscope and time lapse cinematography show that the vacuoles move towards the periphery, forming clear areas. The nucleolus is often found attached to the nuclear membrane and some of the vacuoles and materials may be observed to form the nucleous to the cytoplasm. The nucleolus may be surrounded by a ring of foulgen positive chromatin which may represent the heterochromatic regions of the chromosomes associated with the nucleolus. It is membraneless.

The structure, origin and composition have been ably reviewed by Vincent who was the first to succeed in isolating the nucleolus for study and analysis. The ultra structure has been reviewed by Estable and Sotelo (1955). According to them, the nuceolus is composed of two parts; a filamentous structure called **nucleolonema,** the fibrils being of 80Å in thickness. Electron microscope shows fine granules of 150o distributed on these filamentous structures. The nucelolonema are said to divide and get equally distributed to the daughter cells at the time of cell division (2) The pars amorpha represent the internuceleolonema region which is said to contain RNA. Granules of 100Å resembling ribonucleoprotein particles of the cytoplasm may be found here. Similar granules can also be seen in the nuclear sap. The structure of the nucleolus is strongly influenced by the physiological conditions of the cytoplasm. During mitosis, they undergo cyclical change, disappearing at the prophase and reformed at telophase. It has been established that the nucleoli are formed by specialized heterochromatic regions of chromosomes. These are called nuceolar organizars. Some consider that not only a particular region of these chromosomes but any part of the nucleolar chromosomes is capable of giving rise to the nucleolus. The presence of RNA has been demonstrated by Casperson and Shultz as well as by Brachet. The nucleolus RNA is characteristic and is different from the cytoplasmic RNA. It may resemble the ribosomal RNA in base composition and may contribute to it. The nucleolar proteins are mainly phosphoproteins. Histones have been identified by Casperson. Opinions differ therefore

on the nature of the proteins present in the nucleolus. Regarding the DNA which has been observed by some in the nucleolus, there is controversy; it is believed by most that the DNA observed in the nucleolus may represent parts of heterochromatic regions of the chromosomes which may have penetrated deeply into the nucleoli. Vincent has shown certain enzymes in the nucleolus. Acid phosphatase, nucleoside phosphorylase and DPN synthesising enzymes have been demonstrated. Biochemical analysis reveals that RNA polymerase, ribonuclease are also present.

The function of the nucleolus is very intimately associated with the manufacture of RNA and indirectly with the synthesis of protein. It may also play a role in the synthesis of coenzymes.

CHROMOSOMES

During cell division the chromosomes are much condensed and are readily visible by ordinary optical methods. The giant chromosomes of the salivary glands of certain diptera and the lampbrush chromosomes of amphibian oocytes which are visible in the interphase have been of great value in studying the chromosomal behaviour and structure.

In general the chromosomes have a few common features.

1. All chromosomes have a primary constriction called centromere which represents the point of attachment of the spindle fibres during cell division. It is most clearly seen in metaphase and anaphae. It may have additional metabolic significance since it may divide after replication at a different time from other regions of the chromosome. Its position along the chromosome has been used to classify the chromosomes. (a) Metacentric-when the centromeres are in the middle, (b) Telocentric-when the centromeres are terminal (c) Submetacentric when the centromere is sub terminal in position. The centromere appears like a dense granule surrounded by a clear non staining region. There seems to be no significant internal structure.

Secondary constrictions are usually visible as Feulgen negative or weakly staining gaps in which sometimes a fine chromatin strand which connects the main chromosomes may be visible. The gap in the chromosomes is caused by the presence of a nucleo-

lus at this site during prophase, preventing the coiling of the chromosome. The gap remains when the nucleolus disappears but at metaphase this organelle in sometimes indistinguishable from the rest of the chromosome. Satellite is the region of the chromosome distal to the nucleolar gap. Whether it is large or small depends on the length of the chromosome after the gap. Some chromosomes lack satellites and the chromosomes with the satellites are called Sat-chromosomes.

2. Most chromosomes divide lengthwise and the two halves are called chromatids.

3. When fully stretched each chromatid can be seen to consist of one or more threads called chromonemata, which have beadlike areas of increased density on them. They are known as chromomeres. They vary in size and concentration of chromatin but are constant for any given chromosome among different cells. It has been supposed that chromomeres are tightly coiled regions of the chromosome thread but in the electron microscope image, they appear more as tufts than as tightly coiled knobs. So it is difficult to interpret their structure or to understand their functional significance.

Ultra Structure of the chromosome

It has been very difficult to investigate the structure of the chromosome because of their great complexity. This is largely due to the coiling and super coiling of the microfibrills which form the components of the chromosome. By using materials such as the lampbrush chromosomes, and the chromosomes from the cephalopod molluscs and by using stereoscopic electron microscopy it has been demonstrated very convincingly that the chromosome is composed of fibrils of 100Å. Each of this fibril is composed of 40Å fibrils, which may represent DNA-histone molecules. Ris calls the 100Å fibrils as the elementary chromosome fibrils. A chromatid may consist of about 16 to 32 of these fibrils. The 40Å fibrilsmay be linked by histones or other proteins. The fibrils may run continuously, or they may be in the form of subunits connected by non histone proteins. The individual fibrils may be coiled and may be further coiled again and again.

Number : Even though the number of the nuclei may vary from tissue to tissue, the appearance and behaviour of chromosomes is remarkably uniform. Normally, they are diploid, with a characteristic number, size, shape and longitudinal differentiation. Such a characteristic type is called the karyotype. Deviation from this type is due to evolutionary events, pathological conditions, irregularities in cell division etc.

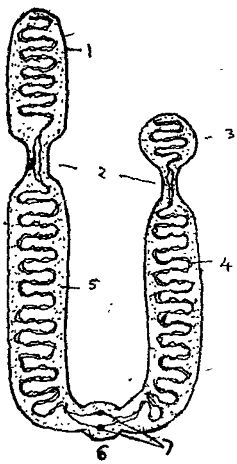


Fig. 144 Chromosome
1. Outer membrane 2. Secondary cons triction 3. Satellite 4. Chromotin thread 5. Matrix 6. Primary constriction (Kineto centre) 7. *Centromeres*

The chromosomes may be differentiated into autosomes and sex chromosomes which are designated as X and Y chromosomes in bisexual organisms.

Chemical composition

The major component of the chromosomes is the DNA which seems to be disposed longitudinally in the chromosome. Histones (proteins) may form cross links. Acidic proteins have been found to occur in large amounts. This substance is some times called chromosomin. The significance of these is at presen not clear.

Function

The chromosome is a repository of genetic information. It contains the hereditary material, the DNA, which is capable of self duplication and also provides the necessary information for protein synthesis.

Genes

These are *the 'elements' of heredity,* and are simply composed of DNA. *With the elucidation of the chemical structure of* DNA by Watson and crick in 1953, a revolutionary insight had been gained into the mechanism of duplication and expression of genes. Har Gobind Khorana has met this challenge and has succeeded in synthesizing the gene for alanine-transfer RNA by chemical means, in May 1970. This feat has

unprecedented implications in medicine. Genetically inheritied metabolic disorders, hormonal imbalances, neural malfunction and mental diseases may be cured in the future by introducing appropriate genes into our cells. There are also dangerous implications, for, unwise use of this technique will cause disaster and destruction.

Giant chromosomes

These are specialised chromosomes, characterised by their large size. Two types are known: (1) Polytene chromosomes-these are found in the salivary glands, Malpighian tubules, gut, trachea and some fat bodies of dipteran larvae. The chromosomes contain many strands. These giant chromosomes have aided in the study of nucelic acid synthesis and for other genetic studies. (2) Lamp brush chromosomes are also very large and occur in the oocytes of the verterbrates. They are characterised by the presence of fine lateral projections or loops which extend from the main axis, giving a brush-like appearance.

CHAPTER XIX

Cell Division

In all living organisms, each cell grows and produces other cells. As the cells of the body are constantly wearing out and leaving the body in the form of excretions, it is essential that the materials lost are replaced and new cells are produced. In a mixed population, the cells in the process of division are usually large ones. To explain this relationship between the size of the cell and onset of division, specific criteria such as the nucleo-cytoplasmic ratio or critical mass have been emphasized. But a more intensive examination of the data makes the hypothesis of casual relationship between cell division and size untenable; for example, the synchronous divisions of the fertilized egg of sea-urchin is not accompanied by periods of growth.

Cells usually divide by indirect cell division, also called Mitosis. In lower organisms, this is the type of division by which the population grows. But in multicellular organisms, division is linked with differentiation.

The reproduction of cells consist of the doubling of all the components of the cells, followed by a division distributing the components equally to the daughter cells. This distribution of materials takes place either by partition of the free organelles or by some other precise mechanism. The whole reproductive cycle is controlled by the nuclear genes.

Cell life cycle: The cell passes through the following stages:

A. Interphase

a. G-1 period of growth consists of that period between the completion of cell division and the beginning of DNA synthesis. Generally the longest and most variable in duration. Active RNA and protein synthesis may occur.

b. S; period: this is the period of replication of genetic material, or DNA synthesis.

c. G2- It is the period of preparation for division. It is a lag period generally observed between the completion of DNA synthesis and the onset of the first recognizable signs of mitosis. RNA and protein synthesis may occur.

B. Mitotic phase

represents the actual division of the cell. It is the shortest period in the life cycle of the cell.

A. INTERPHASE

The combined G1, S and G2 phases are called the interphase. During this period, preparations for mitosis are made and the bulk of the synthetic requirements are fulfilled. The events that occur in interphase may be listed as follows:

1. DNA duplication: This is one of the most important events in interphase by which the DNA content of the nucleus is doubled. From experiments done in the test-tube, it is now known how DNA synthesis may take place in the cell. The double stranded nature of the DNA, wound in the form of a helix has induced scientists to suggest possible ways in which the double stranded DNA could synthesise another DNA double strand, of which each strand would be an exact copy of the original. Taylor,Levinthal Meselson and Sahl and others have contributed much to our knowledge in this respect. DNA replication however is not a requisite to cell division but cell divisions do not take place without a previous duplication of the DNA content.

Replication of DNA

Many models have been proposed to explain the possible ways in which DNA may replicate.

a. Conservative type: According to this model, the original molecule of DNA retains it structural integrity throughout the cell diusion and the parental DNA helix could be passed on to the progeny in intact and one of the daughter cells gets the newly replicated DNA molecules.

b. Dispersive type: According to the model, there is extensive breakdown of the orignal DNA and it is distributed uniformly among the daughter cells.

C. Semiconservative mechanisms: According to this model the parental DNA molecules retain their chemical identity but not their physical identity. The two polynudeotide strands unwind and separate but there is no chemical break down of DNA. The two strands will divide between the two daughter cells. This type of replication is substantiated by labelling experiments and seem valid.

2. Reproduction of centrioles: This is another prerequisite to division. The centriole seems to reproduce by the outgrowth of the daughter centriole by the parent particle and the new particle invariably grows at a right angle to the parent.

3. Increase in the mass of the cell, nucleus and nucleolus

4. Energy liberation for mitosis.

5. Increase in sulphhydryl compounds.

6. Macromolecular synthesis for the spindle formation.

7. All these events overlap to maintin a fludity in cellular, events.

B. MITOTIC PHASE

The sequence of mitotic events are divided into four stages for convenience though it is really a cotinuous process and the phases run into the suceeding phase.

1. Prophase

This is the period of preparation.

(a) This is the stage when there is morphological evidence of the biochemical event of the DNA duplication and the exact number and size of the chromosomes becomes apparent. The tangle of threads are packed into compact masses that can be moved freely and without getting entangled. This is done by coiling and superimposed coiling of the chromatin threads. The inner mechanisms of the coiling in large scale is not very clear. The chromosomes become surrounded by a matrix. The chromosome itself is composed of two strands called chromatids which lie close together and it is not always possible to see the split in the chromosomes throughout its length. The chromatids may be coiled about each other. If the semi conservative model of DNA replication is accepted, the chromatid coil will contain a new

DNA, strand and an old DNA strand. Experiments performed by Taylor with radioactive labels suggest that the strands of chromosome (chromatids) act as a template for the production of another part. It is not known however how many strands of DNA make a chromatid or how this DNA is arranged in the chromosome. Thus while we know something of the DNA duplication at the molecular level, we are not sure of the corresponding structure in the chromosome. The end result however is that a new chromosome is formed which is an exact copy of the one in the mother cell.

It has been noted that upto the time of coiling of chromosome the cell can be prevented from undergoing division by depriving the cell of oxygen or by poisoning its oxidative enzymes with carbon monoxide. But after a certain point-about the time the chromosomes are coiling up-it is not possible to stop the division by blocking oxidations.

(b) Disintegration of nuclear envelope: By this, the barrier between the chromosomes and poles is removed.

(c) Nucleolus gradually becomes smaller and eventually pisappears.

(d) Assembly of mitotic apparatus: Between the poles and around the nucleus. a mass of material gathers which later becomes the mitotic apparatus. Centrioles move apart and establish poles towards which the chromosomes will move. The poles are pushed apart by the growth of fibres that continue to connect the poles together. This is called the central spindle.

(e) The RNA content of the chromosomes increases as well as their phospholipid content.

Thus in prophase, which is long drawn out the chromosomes are consolidated, poles established and substance of mitotic apparatus is gathered and the cell is now set to divide.

2., Metaphase

The centromere or kinetochore which is the anhor point on the chromosome and which has a constant position on each chromosome giving it a particular shape, becomes clearly visible. The spindle fibres are attached to the centromeres

of the chromosomes and direct the movement of the chromosomes. The movement of the chromosomes proceeds in two steps. The first step occurs in metaphase. The paired sister chromatids move into equatorial plane as defined by the poles. At this stage the chromosomes lie in the spindle which is a body between the poles consisting of fibres that connect pole to pole and pole to chromosomes and an unindentified matrix. Asters may be observed to radiate from the pole in the animal cells.

3. Anaphase

The second step in the chromomal movement is seen in this stage. The centromere divides and the chormatids split apart and move to the poles. The separation of sister chromosomes and their migration toward the poles have been described

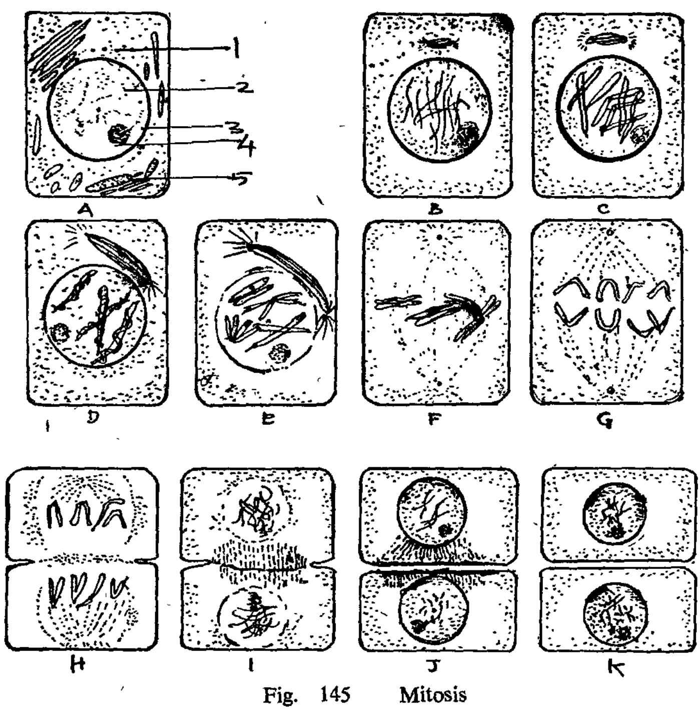


Fig. 145 Mitosis

1. Cytoplasm, 2. Nucleus, 3. Nuclear membrarc, 4. Nucleolus, 5. Mitochondria.

in detail and have also been measured, by microscopic studies as well as by motion picture camera. The distances traveled by the chromosomes is 5 to 25 microns. Velocity is about 1 micron per minute. The chromosomes move in straight lines usually converging at the poles. Often as the chromosomes move towards the poles, the poles are further separated from the other. It is not known with certainty how the movement of the centromeres is conrolled nor how each centromere is attached to the spindle fibre.

The mitotic spindle plays a crucial role in this stage. It has been described as a gel, transparent and excludes large particles such as mitochondria. The molecular components of the spindle tend to be oriented along the pole to pole axis. The fibres are fine and straight, usually double and sometimes occur in bundles, running from centromeres to poles. These filaments shorten as the chromosomes move to the poles and lengthen when the poles move apart. Studies from isolated mitotic apparatus give a clue as to their molecular structure. It is chemically unstable when removed from the cell. Therefore it seems reasonable to suspect that there is a stabilizing factor in the internal environment of the cell. Sulphur bonds play an important role in holding the mitotic apparatus together and a compound incorporating sulphur to sulphur bonds seem to be involved. It has been shown that the mitotic apparatus contains a great deal of protein. It also contains RNA much of which is associated with a major protein. There is also considerable amount of lipids. According to earlier experiments, chemical bonds between sulphur atoms on neighbouring protein molecules were considered important for holding mitotic apparatus together.

4. Telophase

Once the chromosomes have been separated into two groups, they get organised into two interphase nuclei. They become surrounded by nuclear envelops. Very little is known of the details of this construction. Nucleolus is reformed.

Now eytokinesis or division of the cytoplasm takes place. This is one of the remarkarle events. Many theories have been proposed to explain the mechanism of cytoplasmic division.

Division does not depend on the chromosomes but on the mitotic apparatus. In plant cells cytokinesis involves the laying down of a cell plate between the two reorganizing telophase nuclei. In animal cells cytokinesis consists of an equatorial constriction which leads directly to the separation if the pressure of neighbouring cell is absent.

Initiation of mitosis

There are many possible factors which trigger the mitosis, such as doubling in the mass of the cell, upset in the surface to volume ratio of the cell, doubling of DNA content, activity of the nucleolus etc.

MEIOSIS

Meiosis is the type of cell division which exclusively occurs in the gonads during gamete formation. The factors which initiate meiosis are not fully known. The relative amount of RNA and DNA is one of the possible factors.

The essential characters of meiosis are

1. Pairing and chiasma formation in the chromosomes,

2. Reduction in the number of the chromosomes. The events of the meiosis occur in two stages, called Meiosis I and II. Autoradiographic evidence has clearly demonstrated the completion of DNA synthesis during the meiotic interphase. It has also been demonstrated that the duplication is by semi-conservative mechanism like in mitosis. Almost all the RNA synthesised is of chromosomal origin, very little by nucleoli. In the mitotic nucleus on the other hand the bulk of the RNA is synthesized by the nucleolus. In addition the RNA is synthesized during the later stages of meiotic prophase when nucleolus is not present. Ribonucleic proteins may also be synthesized during the different stages of prophase in meiosis, the most active periods being leptonema and pachynema.

Meiosis I

1. Prophase I

It is of extremely long duration during which there is an

increase in nuclear volume by gradual modification in nuclear structures. It is divided into 5 substages.

a. **Leptonema:** (Leptotene): The chromosomes are long and are greatly extended and uncoiled. They show maximum extension. In late leptonema, the chromonema are of uniform diameter thrown into large number of coils of small size. Duplication of chromosomes may occur in this stage in some cells. In many animal cells, the free ends of the chromosomes are attractee to the side of the nucleus nearest the centrosome and the body of the chromosomes extends in a loop in the interior. The chromosomes then present a bouquet like appearance. The chromosomes are said to be polarized. The nucleolus increases in size, in volume and in RNA content, between leptonema and the next stage (Zygonema). At the end of leptotene, chromosomes are shorter in length and wide in diameter due to increases in the diameter of the spirals.

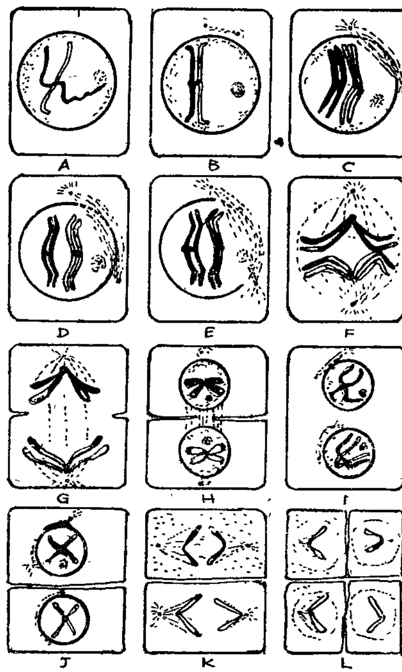


Fig. 146 Meiosis

A. Leptotene, B. Zygotene
C. Pactrytere, D. Diplotere, E. Diakinsis
F. Meta Plese, G. Anaphase. H. Telopore
I. Prophax II, J. Metapiaer, II,
K. Anaphase II. L, Telophase II

b. **Zygonema:** It is one of the most important stages of prophase I. Two similar chromosomes (the homologous chromosomes) one from the paternal and the other from the maternal origin, are bought into apposition at one or several places (Synapsis). After the first contact, pairing continues in a zipper like fashion along the chromosomes. The pairs so formed are called bivalents. Since each chromosome is formed of two chromatids, each

bivalent consists of 4 chromatids or tetrads. The reason for pairing is not clearly understood. This stage is of long duration

c. **Pachynema:** The pairing is completed and the number of pairs of chromosomes is now half the diploid number. The chromosomes become shorter and thicker about 1/4th or 1/6th of leptonema. Sometimes the apposed homologues are twisted about one another. Crossing over occurs at this stage and bivalents exchange their segments by chromosomal break and union resulting in crossshaped figures called chiasmata. The number of chiasmata depends on the length of the chromosome and the time of occurence is variable. The localisation of chiasma seems to be under genetic control.

d. **Diplonema:** This stage is characterised by the tendency for paired chromosomes to fall apart. The repulsion between centrometres is very evident and the chromosomes are attached only at the chiasma. Asa result, the bivalents appear as loops. In the late diplotene, terminalization occurs by which chiasmata move towards the chromosome ends.

e. **Diakinesis:** The chromosomes become still shorter due to further coiling and individual chromatids cannot be identified. Terminalization is completed (if not already completed) and the bivalents migrate to the periphery of the nucleus where they lie in close association with the nuclear membrane and widely separated from one another. The last remnant of the nucleolus disappears. Nuclear membrane breaks down and the spindle is formed by the centrioles.

2. Metaphase I

The bivalents are now established on the spindle. The two homologous centromeres of each biavlent lie towards the poles while the arms of chromatids are directed towards the equator. The spindle fibres which are connected to the centromeres are called chromosomal fibres.

3. Anaphase I

The tetrads become separated into two dyads. The chiasma slips off at the free ends, thus disengaging paired homologues.

A dyad consists of two chromatids each made up of two arms, so it appears as a double V or double J. The four arms do not lie closely appressed during anaphase I but diverage as if they were mutually repelling one another.

4. Telophase I and Interphase

The chromosomal dyads reach their respective poles. Nuclear membrane is formed and chromosomes elongate, loosening the coils. But they do not fully extend as the interphase is short. There is no duplication of the chromosomes and dyads reappear unchanged in Prophase II.

5. Prophase II

It is unspectacular and there are no unusual phenomena. Superficially, it resembles ordinary somatic division. The two chromatids look like 'x', connected by centromeric regions with arms widely apart. There is no relational coiling, but the chro-nemeta are not completely uncoiled. The generic constitution of the two chromatids of each dyad depends on the kind of and members of cross over. Nuclear membrane and nucleolus begin to disappear.

6. Metaphase II

This stage is of very short duration. The chromtids become lined up with the centrometres on the equator. The centromeres lie along the equator while their arms are separated out. Centromere becomes functionally double.

7. Anaphase II

The chromosomal fibres arise from the centrioles and the two chromatids move to opposite poles.

8. Telophase II

The daughter chromosomes formed of the chromatids uncoil and nuclear membrane and nucleolus appear.

Cytokinesis follows, resulting in four daughter cells, each with reduction in the chromosome number and recombination achieved.

Comparison between Mitosis and Meiosis

	Mitosis	Meiosis
1.	This cell division is called indirect division.	This is called reduction division.
2.	This takes place in all growing parts of plants like shoot tips, root tips etc. and in the body cells of animals.	This takes place only in the reproductive organs, during gametogenesis.
3.	As a result of Mitosis, two daughter cells, which are identical quantitatively and qualitatively are created.	As a result of Meiosis four cells, each different from the other in chromatid content and all having only half the number of chromosomes as the mother cell are formed.
4.	Prophase of Mitosis is of short duration.	Prophase is a prolonged affair, *i e.* takes a longer time.
5.	There are no substages in prophase.	There are 5 substages in prophase, namely, Leptotene, Zygotene, Pachytene, Diplotene and Diakinesis.
6.	Chromosomes do not pair during prophase.	Homologous chromosomes pair during zygotene.
7.	There is no exchange of genetic matter between chromosomes.	There is an exchange of segments of chromatids between homologous chromosomes.
8.	Chromosomes split up into two chromatids, completely. The separation starts with the splitting of centromeres during Metaphase.	Chromosomes do not split entirely. The centromeres do not divide.
9.	During Anaphase chromatids are separated and pulled towards opposite poles.	Chromosome pairs are separataed during Anaphase. Chromatids are not separated.
10.	Only two daugher cells are formed at the end of Mitosis.	Four cells are formed because there is a second division called Meiosis II.

CHAPTER XX

Differences Between Animal and Plant Cells

1. In most plant cells, the cell has a semi-rigid, laminated, external, covering. It is secreted by the cell and gives protection and support, covering the living plasma membrane beneath. In some plants, it contains minute pits or canal like plasmodesmata, that connect adjacent cells. In plant cells it is made primarily of cellulose. Other compounds such as fatty substance, tannins proteins, gums and mucilages also may be present in the cell walls. The cell walls provide tensile strength and protection to the cell and to the plant as a whole. It counteracts the osmotic pressure being developed by the cell inclusions. They also serve to provide sites for enzymatic activity and a passage for materials for growth of the cells. The cell wall also serves as a barrier preventing the penetration of several micro organisms into the cytoplasm.

2. Cytoplasmic vacuoles are fewer and larger in plants. They may function for storage, for transmission of materials and for help in maintaining the internal pressure in cells.

3. Plastids are organised bodies, usually spherical oval or ribbon shaped, and are present in certain plant cells. Three types are found in plant cells which include (a) chloroplasts which contain green chlorophyll and absorb radiant energy to photosynthesize foods (b) chromoplasts which are usually yellow, orange or red and occur in flowers and fruits (c) leucoplasts which are colourless and may store energy rich starch as in the potato tuber. Rhodoplasts are red coloured plastids found in Rhodophyta and Phaeoplasts which are yellow plastids found in brown algae, diatoms and dinoflagellates.

4. Centrioles are not found in higher plants, though it may be present in certain lower plants such as brown algae.

CHAPTER XXI

Biosynthesis of Protein

All the reactions involved in synthesis are mediated by enzymes and the key to the increase in cellular material associated with growth is the synthesis of proteins. It has been of greatest interest to man and has been thoroughly investigated. Brachet and Caspersson have shown beyond all doubt that RNA is involved in the protein synthesis. Three major processes are involved in the protein synthesis.

1. Flow of matter in the form of aminoacids in the cytoplasm.

2. Flow of energy: Aminoacids cannot polymerize by themselves but must be activated, which involves ATP activating enzymes and an adaptor molecule (tRNA).

3. Flow of information: Information in the DNA base sequences in the form of triplet code, is transcribed by mRNA. Each aminoacid is able to recognise a sequence of nucleotides through the mediation of tRNA.

The synthesis of protein can be described in the following steps:

1. DNA, with the aid of DNA polymerase, elaborates a RNA molecule whose base composition is complementary to the DNA template, thus imparting the genetic information contained in DNA to this mRNA. (transcription). The mRNA leaves the nucleus and moves to the ribosomes and may activate them to form poly ribosomes.

2. In the meanwhile, aminoacids in the cytoplasm are activated by reaction with ATP in the presence of an enzyme, forming an aminoacid-adenosine monophosphate complex (aminoacyl AMP-enzyme couple)

Aminoacid + enzyme + ATP Enzyme (a,a AMP) + PP

3. At the same time, an acceptor site for the aminoacid on the tRNA (transfer RNA) is prepared by its reaction with cytidine triphosphate (CTP) and adenosine triphosphate (ATP).

$$RNA + 2CTP \rightleftharpoons RNA \overset{c}{\checkmark}\overset{c}{\checkmark} + 2PP$$

$$RNA \overset{c}{\checkmark}\overset{c}{\checkmark} + ATP \rightleftharpoons RNA \overset{c}{\checkmark}\overset{c}{\checkmark}\overset{c}{\checkmark} + PP$$

(Phosphorylated tRNA)

4. The activated aminoacid complex is next attached to the phosphorylated tRNA and AMP is released.

$$RNA\overset{c}{\checkmark}\overset{c}{\checkmark}\overset{c}{\checkmark} + E\ (a,a \wedge AMP) \rightleftharpoons (RNA)\ \overset{c}{\checkmark}\overset{c}{\checkmark}\overset{c}{\checkmark}$$
$$—a.a + AMP + Enzyme$$

Thus 20 such tRNA occur, one for each aminoacid.

5. To be formed into protein, the aminoacids are carried by the tRNA to the ribosomes where the activated aminoacid becomes attached to the mRNA. In the presence of guanosine triphosphate what happens on the surface of the ribosomes is highly speculative. The recognition of appropriate site of attachment on the polyribosome is contained within the tRNA and not aminoacid. The recognition site in tRNA is called the anticodon and it is complementary to the mRNA in the polyribosome. The codon then directs the formation of hydrogen bonds between itself and the anticodon, a process which may require NH_4 or K^+. The attachment of aminoacyl tRNA to the polyribosome is complex. The mRNA are bound to the 30S subunit of the ribosomes while the aminoacyl tRNA is in some manner attached to the 50S unit.

6. Growth of proteins is by stepwise addition of single aminoacid at the N-terminal amino ends in the polyribosomes and the growing poly-peptide chain is set free. Peptide bonds are formed connecting the aminoacids, the sequence of arrangement of the aminoacids being determined by the template provided by the mRNA. Meanwhile the tRNA is liberated as the aminoacid joins in the formation of protein.

The question is how the nuclear DNA gives to the mRNA its coded information as to what kind of protein to be made.

A triplet genetic code consisting of the base sequence in the DNA has been formulated from various experimental evidences and it has been shown that the base sequences in DNA determine the aminoacid sequence in the protein. A dictionary has been compiled showing the code for the aminoacids.

Protein synthesis takes place in nucleus also to some extent.

The various steps in protein synthesis outlined above are based on experimental evidences from fractionation studies and cell free systems and are based on a few crucial pieces of such experimental evidence, such as:

1. The demonstration that DNA carries genetic information in bacteria. In 1944, Oswald Avery and his co-workers demonstrated that DNA was the agent responsible for transformation in bacteria. Pneumococci (Streptococcus pneumoniae) are enveloped by capsules of polysaccharide and present a smooth appearance. They are called S colonies. They breed true. Mutations occur in the colony and the mutants form a colony of rough apperance and they seem to have lost the ability to manufacture capsules. They are called R colonies and are nonpathogenic. In an experiment conducted by Frederick Griffith (1928) it was shown that when a mixture of living R cells and killed and therefore non virulent S cells, were injected into mice living S cells were recovered from the mice. It was interpreted to mean that some substances had been liberated from the dead S cells which had **transformed** the R cells and made them capable of synthesizing the capsule. It is these substances or **'transforming principle'** that has been identified as DNA, proving that DNA is the genetic determinant. Many other experimental evidences have accumulated since then to confirm the view.

2. The demonstration that RNA synthesis in the cell is governed by DNA.

3. The demonstration that the sequence of aminoacids in a polypeptide is determined by a sequence of nucleotides in a molecule of RNA.

Though these processes have not been shown experimentally in all cells, there is enough evidence for the hypothesis that it is an universal mechanism for the genetic control of cellular activities.

Other synthesis

For synthesis of carbohydrates, the exact reverse of catabolic processes in respiration takes place and along with protein become a structural and functional components of the cell, Plant cells prepare carbohydrates from raw materials from the environment (Autotrophs). They do not build up great quantities of simple carbohydrate in this way but they serve as raw materials for further synthesis of organic compounds.

Photosynthesis

Chloroplasts are the cytoplasmic factories where photosynthesis takes place in the cell. The chloroplast itself is composed of numerous granules or **grana** which are the functional units of the chloroplasts. In blue green algae the grana may be diffuse. A green cell may possess a few to about 80 chloroplasts and they reproduce within the cells. Chloroplasts contain mostly green pigments (Chrolophyll). The chrolophyll molecule consists of 4 complex carbon N rings, which in turn are combined into a larger ring, the 'head of the molecule'.

The events in photosynthesis may be summarized as follows:

1. Photolysis

(a) Chlorophyll molecules absorb a portion of the light energy.

(b) Light energy, thus trapped in the chlorophyll molecule splits the water into hydrogen and oxygen. The oxygen may escape into the immediate environment of the plant, and hydrogen is captured by hydrogen acceptors and finally delivered to the complex cycle of carbondioxide fixation.

2. Carbondioxide fixation

The carbondioxide combines with hydrogen to form carbohydrate. This reaction is a complex one and occurs through a cycle of reactions.

The following steps may be said to occur.

(a) CO_2 is taken up by a 5C compound, already present in the chloroplast, called ribulose diphosphate (RDP), and with

the addition of water produces two 3C molecules of a compound called phosophoglyceric acid. (PGA).

(b) PGA is then converted into phosphogleceral dehyde (PGAL) by losing an oxygen atom (which is joined to hydrogen from photolysis). As 3 CO_2 molecules enter the cycle, one molecule in each turn there are three turns of the cycle. So 6 PGAL molecules are produced in this fashion per chlorophyll molecule. 5 of the 6 PGAL molecules are changed into 3 of RDP which then undergo the cycle of combining with CO_2 and H_2O, once again. One molecule of the PGAL is made available to the plant.

3. Thus PGAL is the end product of phtosynthesis, not glucose. It may be converted into glucose and condensed to form starch or may be utilized as fuel or may be exported to other cells. For conversion into glucose, two molecules of PGAL are needed to manufacture one molecule of glucose. So the CO_2 fixing cycle must go around 6 times before one glucose molecule can be obtained.

OVERALL EQUATION

Solar energy $+ 6CO_2 + 12H_2O \longrightarrow$ (Chlorophyll)
$C_6H_{12}O_6 + 6H_2O + 6O_2 \uparrow$

CHAPTER XXII

D.N.A. and R.N.A.

Basic Organisation of these Macro molecules

Every cell contains a Nucleus. The Nucleus has inside it a network of thread like structures called the chromatin reticulum. This network condenses and organises itself as indivdual chromosomes during the cell division. These undergo ia duplicating process during every Mitotic division so that the new daughter cells have the same *component* of chromatin material, both quantitatively and qualitatively. The chromosomes are known to be the seats of genes or the factors that govern the heredity. Besides it is the chromosome that governs chemical activities of the cell. Each chromosome has a definite number of genes in a particular sequence. Such an important item in the cell needs to be understood well, if we are to comprehend the wonders of the living world. The number and shape of chromosomes for any given species of living organism is constant. For exmple the human body cells have 46 chromosomes each.

Chemical analysis of the chromosomes show that they are made of a nucleic acid known as D.N.A. or Deoxyribose Nucleic Acid. What is D.N.A.? It is a macro molecule of certain chemical principles.

Each molecule of the DNA consists of sugars, phosphates and 4 types of nitrogenous bases. The sugars are deoxyribose sugars. The sugar is a pentose sugar (5 carbon sugar). The position of the elements are best seen in the diagram below:

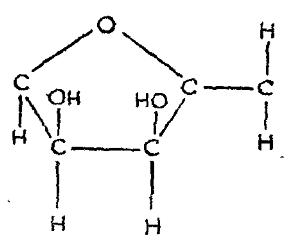


Fig. 147 Sugar

The phosphates are the connecting links between the sugars. The phosphate is represented by

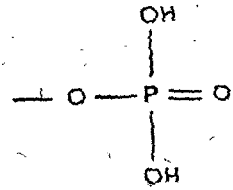


Fig. 148 Phosphate

The 4 types of nitrogenous bases are Adenine, Guanine, Thymine and Cytosine. The adenine and guanines are known as Purines. The thymine and cytosine are called pyrimidines. These nitrogenous bases are usually attached to the sugars in the Nucleic Acid. The chemical nature of these nitrogenous bases are shown below:

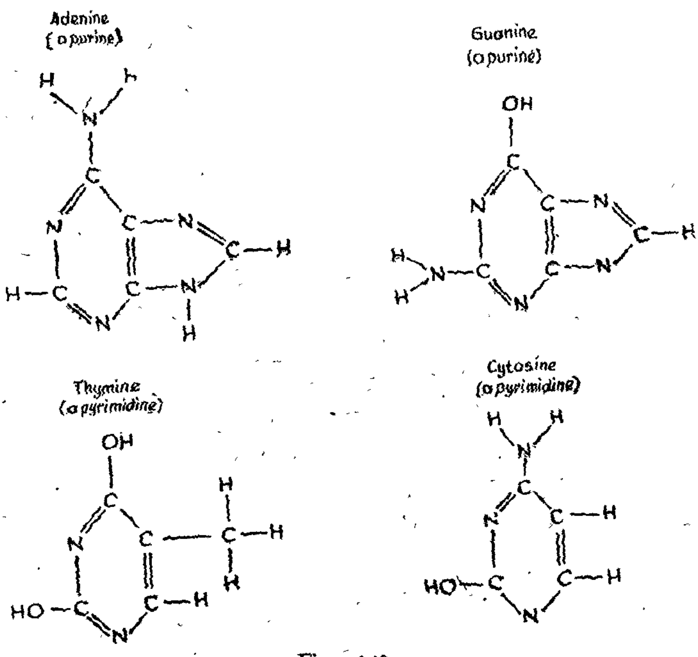


Fig. 149

The DNA molecule is a macro molecule having a molecluar weight ranging between 30,000 to several millions. The nature of the molecule was first demonstrated by a model conceived by

two scientists by name J. H. Watson & F. H. Crick (Nobel prize winners) They believed that the DNA molecule is like a twisted ladder. The two rails of the ladder are formed by the sugar and phosphates arranged in a linear fashion alternately. The sugars in the two rails of the ladder are just opposite and they are connected across by a rung of the ladder. The rung is formed by the nitrogenous bases. As a rule, a purine and a pyrimidine join together to form a rung. Not only that, but it is always the purine Adenine and the pyrimidine, Thymine that form a rung. Similarly the purine Guanine joins a pyrimidine Cytosine to form a rung. These rungs connect the sugars of the two rails of the ladder. The connection between a purine and a pyrimidine is formed by what are known as hydrogen bonds. The Adenine Thymine rungs are connected by 2 hydrogen bonds and the Guanine Cytosine rungs by 3 hydrogen bonds. So the Guanine Cytosine links are a little more stronger. The sequence of the nitrogenous bases forming the rungs can be modified into innumerable ways and that accounts for the millions of different DNA molecules forming variety of organisms.

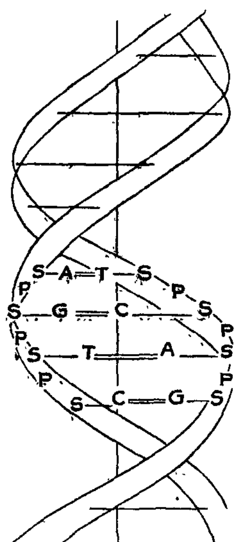


Fig. 150 D. N. A. Structure

The twisted ladder like DNA molecule is referred to as a double helix. The width of the helix is about 20 Å (1 Å Angstrom is 1/10000 of a micron. 1 micron is 1/1000 of a m.m.) One twist of the helix has about 10 rungs across and has a length of about 34 Å.

The unit of one sugar and one nitrogenous base, together is known as a nucleoside. There are therefore 4 types of nucleosides in the DNA, equal to the combination of the sugers with the 4 types of the nitrogenous bases. Each nucleoside along with a phosphate comes to be called a nucleotide. An average DNA molecule has thousands of nucleotides. A gene is a sequence of nucleotides the minimum number of them being three. While the DNA is found in the Nucleus the other Nucleic Acid namely RNA is found both inside the nucleus and outside in the cytoplasm.

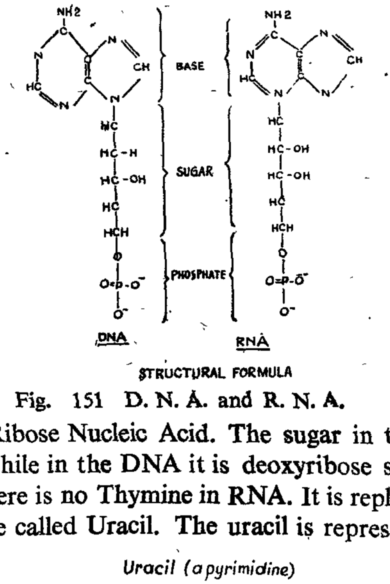


Fig. 151 D. N. A. and R. N. A.

RNA is Ribose Nucleic Acid. The sugar in this molecule is Ribose sugar, while in the DNA it is deoxyribose sugar (i.e.minus-OH). Besides there is no Thymine in RNA. It is replaced by another nitrogenous base called Uracil. The uracil is represented as below:

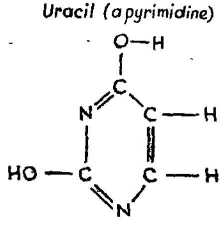


Fig. 152

The RNA molecule is not like a double helix but like a loop.

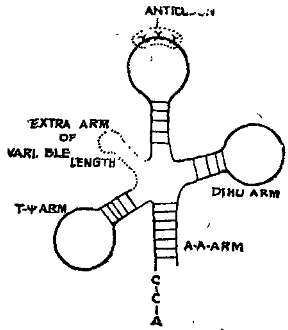


Fig. 153

The RNA molecule is mainly of 3 types. They are (1) Transfer or soluble RNA (2) Template RNA or messager RNA and (3) Ribosomal RNA.

The RNA plays an important role in protein synthesis. (Refer chapter on Protein Synthesis)

CHAPTER XXIII

A.T.P. and Its Role in Cellular Activity

All activities of life require energy. This energy source can be traced ultimately to solar energy. This is trapped by the chlorophyll of the plants and stored as carbohydrates and fats. That is glucose is built up from raw materials like CO_2 and water with the help of solar energy. This glucose is polymerised into starches and sugars (Carbohydrates) and fats. For getting the energy for life activities, glucose is the starting point. A molecule of glucose is oxidised to release about 673 kilo calories of energy. The energy is in the form of high energy bonds between phosphates. The 673 kilo calories of energy is obtained as 38 ATP molecules in the living cells. In other words the ATP molecules are current coins of chemical energy.

What is an ATP molecule? Each ATP molecule has a nitrogen base called Adenine (ref. chapter on DNA), a ribuloes

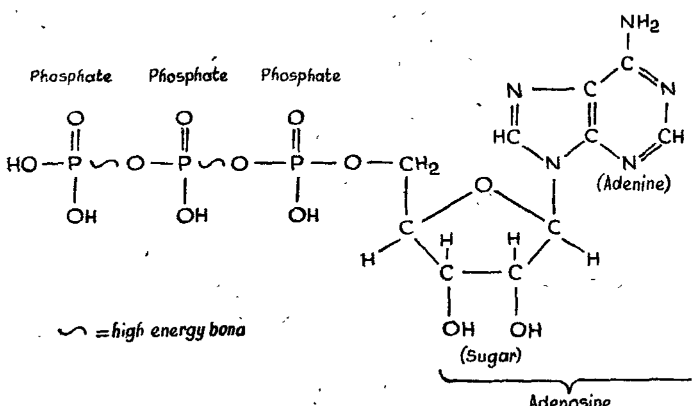


Fig. 154

sugar and three phosphate groups. The Adenine and sugar together form Adenosine, which is a nucleoside. The nucleoside

and one phosphate group forms a Nucleotide. The other two phosphate groups are attached to the first phosphate group of the Nucleotide by what are called high energy bonds.

Kreb's Cycle

Glucose ($C_6 H_{12} O_6$)

Glucose + Phosphate = Glucose Phosphate

↓

= 2 molecules Pyruvic acid + 2 ATP

$C_6 H_{12} O_6 \longrightarrow 2\ (CH_3\ COCOOH) + 2\ ATP$

Pyruvric Acid + Coenzyme A

= Acetyl Coenzyme A

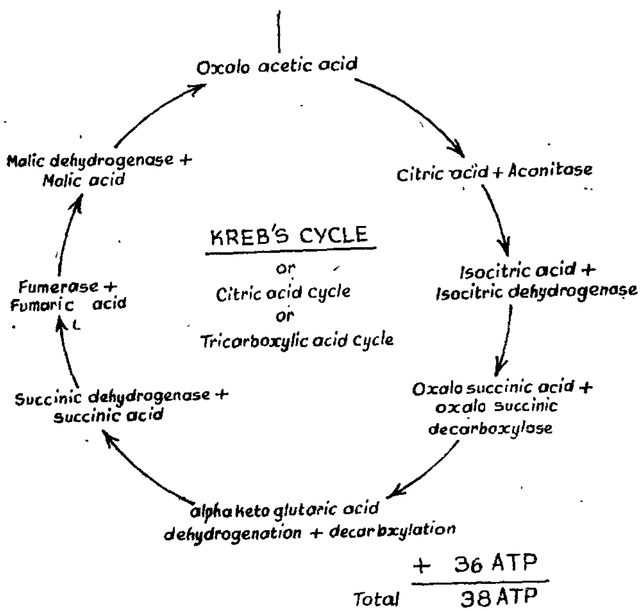


Fig. 155

ATP is built up during Photosynthesis. During the photolysis of water, an electron is released, which is returend to chlorophyll through Cytochromes in the cell. During this electron transfer, an ADP (Adenosine di-phosphate) molecule becomes

phosphorylated to an ATP molecule (Adenosine Tri-Phosphate). This is called photophosphorylation. Besides glucose is built up as a result of photosynthesis, which can be oxidised back to yield ATP molecules.

ATP is obtained during the break down of glucose molecule by respiration. One molecule of glucose yields 38 ATP molecules during the respiratory process. The break down of glucose is shown in the 'kreb's cycle.' The first step (i.e.) glycolysis (breaking down of glucose to pyruvic acid) takes place in cytoplasm and other steps in Mitochondria. The breakdown is effected by a series of oxidation reduction processes, in what is known as electron transport system, involving many substances or enzymes like DPN (Diphospho pyridine nucleotide) Riboflavin coenzyme and cytochromes.

CHAPTER XXIV

Tissues

A Tissue is a group of cells, which are all, similarly modified, in order to carry out a particular function. Though an organism is made of numerous cells, all the cells are not similar Cells undergo structural changes in order to suit the special function performed by them. This kind of structural modification to suit the functional needs is a consequence of division of labour and it is called differentiation. Such a group or assembly of similarly modified cells with a common function is called a tissue (e.g.) Epithelium, Muscle, Nerve, Connective tissue, Cartilage, Bone, Blood and Germ cells.

Epithelium: This is usually a protective tissue. It is formed by a layer of cells above which, there is a free surface. The free surface may be external or internal. The cells are all arranged in a single layer on a gelatinous substance called Matrix or basement membrane. Blood capillaries and nerves stop in the matrix. So the cells of the epithelium have to take whatever they want only from the Matrix. The epithelia are of different types:-

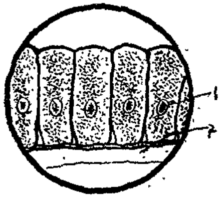


Fig. 156
Columnar epithelium
1. Nucleus. 2. Matrix

1. Columnar epithelium: This type of epithelium is found on the inner wall of the oesophagus, intestine and in some ducts. The cells of this epithelium are long and pillar like. Their inner ends are narrow. They are arranged vertically on the Matrix. This epithelium helps in absorption.

2. Ciliated epithelium: This type is found on the inner wall of the trachea in Man. The cells of this epithelium have minute hair like cilia on their free outer surface. The cilia exhibit vibratile movements. The cilia help to move particles on the surface gently. In other words, they help to keep the surface clean.

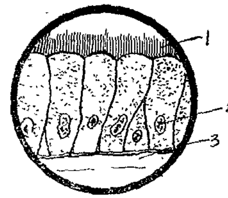


Fig. 157
Ciliated epithelium
1. Cilia 2. Nucleus
3. Matrix

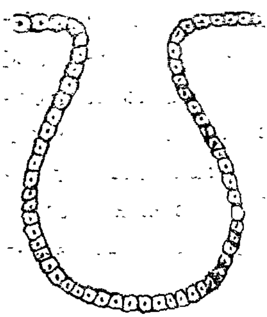


Fig. 158
Glandular epithelium

3. Glandular epithelium: This type is found on the inner wall of the stomach and in many glands. The cells of this epithelium are capable of secreting fluids like mucus or enzymes. Some of the cells are having cavities, resembling wine glasses. These are called goblet cells. The glands may be on the surface, or invaginated like a test tube, or it may be a very much branched gland.

4. Squamous epithelium: This is found on the inner wall of the lips. The cells are reduced to thin plate like or scale like structures. The cells are arranged on the matrix like the stone or tiles on a pavement. So it is also called a pavement epithelium. The main purpose is protection to the tissues below.

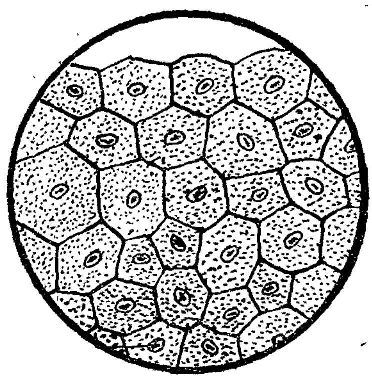


Fig. 159
Squamous epithelium

5. Stratified squamous epithelium: This is found in the corneous layer of the epidermis of the skin. The cells are also

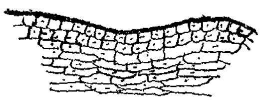


Fig. 160
Stratified squamous epithelium

dead and reduced to thin plate like scales. But there are several layers of these cells on the matrix.

6. Germinal epithelium: On the wall of the gonads (Testis or Ovary) epithelial cells give rise to reproductive cells. So it is called germinal epithelium.

7. Sensory epithelium: The sense organs like skin, tongue, nostrils etc. have a layer of sensitive cells which convey impulses to the brain and help us to understand the various senses. So these cells form the sensory epithelia.

Muscle tissue: The muscle cells are adapted for bringing about movements. The cells are long and contractile. They can contract and relax. This is a physico-chemical reaction. It is also a reversible reaction. The muscles are of 3 types.

1. Voluntary muscles: The muscle cells are long and cylindrical. The nucleus is placed towards one side of the cell. The cell is enclosed in a protective sheath called sarcolemma. The muscle cell shows many dark and light bands across its length. These are known as striations or stripes. Hence these muscles are also called striated or striped muscles. The muscle cells are found in bundles called fasciculi. Many fasciculi are bound into a muscle. These muscles are usually found attached to the skeleton. So they are called skeletal muscles. These muscles work under the control of conscious will. Hence they are called voluntary muscle. (eg.) Biceps, triceps etc.

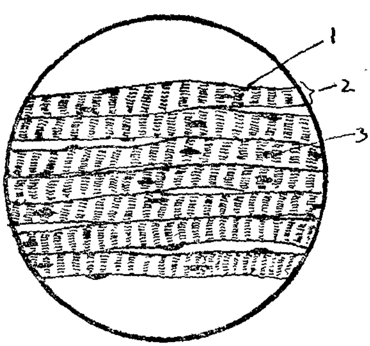


Fig. 161
Volumtary muscle
1 Striations 2. Muscle cell
3. Nucleus

2. Involuntary muscle or smooth muscle : These muscle cells are spindle shaped, with a *middle* broad portion and tapering

ends. The nucleus is in the centre. There are no stripes of striations. There is no sarcolemma. The muscle is called unstriped or non-striated muscle. These muscles are not under

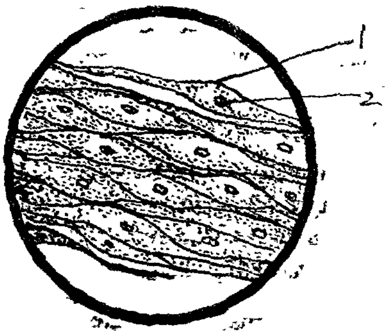


Fig. 162
Involuntary Muscle
1. Cell 2. Nucleus

the *control* of will. So these are called involuntary muscles (e.g.) muscles on the wall of the alimentary canal, urinary bladder etc.

3. **Cardiac muscle:** The wall of the heart is made of a special type of muscle cells. The cells are branched and connected like a net-work In each cell, the nucleus is towards one side and there are dark and light bands across the cell. In these respects it is like voluntary muscle. There is no sarcolemma and these muscles are not under the control of will. Hence it is like involuntary muscle. But the heart muscle cells are different from both voluntary and involuntary muscles in being branched.

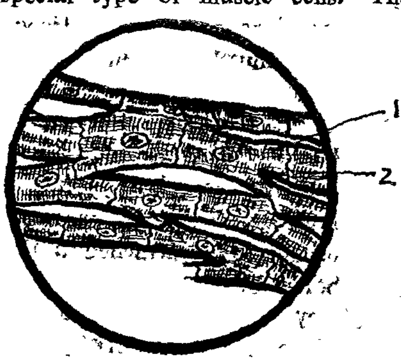
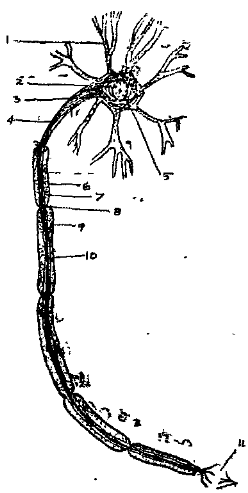


Fig. 163
Cardiac muscle
1. Cells 2. Branch

Nerve cells : The nerve cells are also called **Neurons.** These cells are highly modified for communication inside the body of an animal. The cells are extremely adapted and sensitive. The brain, spinal cord and the nerves are all made of this type of cells. The nerve cell has an irregular cytoplasm with nucleus in the centre. The cytoplasm contains many granules called Nissl's granules (RNA). The corners of the cell are produced into small tree like branches called dendrites. Nerve messages enter the cell only through the dendrites. From one corner of the cell, arises a long fibre, called Axon fibre. Messages leave the cell through the axon fibre.



1. Dendrites
2. Nucleus
3. Cytoplasm
4. Axon fibre
5. Nissl's granule
6. Neurilemma
7. Myelin sheath
8. Node of Ranvier
9. Nucleus
10. Axon fibre
11. Synapse

Fig. 164

Nerve cell

This fibre is covered by 2 coats. The inner coat is called Myelin sheath or sheath of Schwann. The outer coat is called Neurilemma. This coat is constricted at intervals. These constrictions are called nodes of Ranvier. The free end of the axon fibre is branched and it always joins the dendrite of another neuron. At the junction, a small gap called synapse is present. In this gap, messages are transmitted chemically.

A nerve is a bundle of axon fibres. The nerve cells or neurons are present only in the brain, spinal cord and in the ganglia.

Connective tissue: The many tissues in an organ are kept in, position and bound together by the connective tissue. This tissue has few large cells which secrete a gelatinous matrix.

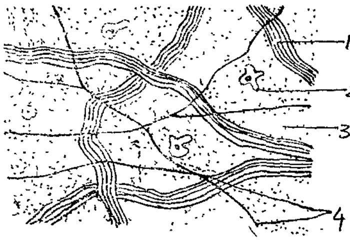


Fig. 165

Connective tissue

1. White fibres 2. Cell 3. Matrix 4. Yellow fibres

This matrix is strengthened by bundles of unbranched white fibres and single branched yellow fibres. These fibres are produced by special cells called fibrocytes. The fibres are made of a material called collagen.

Cartilage: The Cartilage is found at the junction of two or more bones. The external ear and the tip of our nose are supported by cartilage. The cartilage is a tough, but at the same time flexible tissue. It acts as a cushion at the junction of bones.

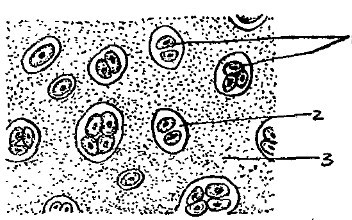


Fig. 166

Cartilage

1. Cartilage cells 2. Lacuna 3. Matrix

The cartilage cells appear in groups of twos and threes. The cells secrete a matrix made of chondrin. Because of the matrix the cells appear to be inside cavities called lacunae. There are three types of cartilages (1) Hyaline cartilage; when the matrix is free of fibres and salts. (2) Fibro Cartilage when the matrix shows fibres running across, and (3) Calcified cartilage, when the matrix is full of calcium salts to give it additional strength.

Bone: The bone is a hard structure in the centre of which there is a soft tissue called red marrow of bone. (New red

blood corpuscles are produced in the marrow). The hard part contains many small cavities known as Haversian canals. Each Haversian canal contains a blood capillary. The Haversian canal is surrounded by many small cavities arranged conecntrically. These are the lacunae containing the bone cells or osteoblasts. The bone cells are all connected with each other and with Haversian canal by minute tubes called canaliculi. The substance in between the canaliculi is the matrix. It is fully impregnated with calcium salts. Bones give strength, shape and support to the organisms.

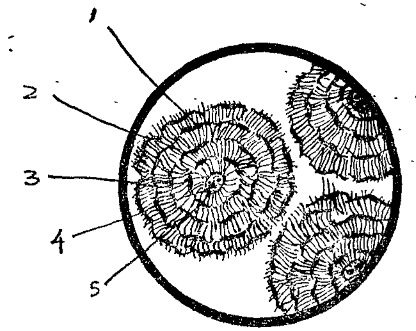


Fig. 167 Bone

1. Bone cell 2. Canaliculi 3. Matrix 4. Haversian canal 5. Capillary

Blood: Blood is a tissue made up of a liquid matrix, called plasma, in which groups of cells called corpuscles float. The plasma is a pale yellow watery liquid containing about 95% of water. It also contains digested food (glucose and aminoacids) metabolic waste (urea, uric acid) gases (O_2 and CO_2) hormones, (secretions of ductless glands) antibodies, inorganic salts(chlorides carbonates, sulphates of Calcium, Sodium, Iron, Magnesium, Iodine, Potassium etc.) and blood proteins. (serum albumen, serumglobulin, prothrombin and fibrinogen). The plasma being a liquid is useful for distribution of food, O_2, heat etc.

The corpuscles are of three types namely (1) Red corpuscles or erythrocytes (2) white or colourless corpuscles or leucocytes and (3) platelets or Thrombocytes. The red corpuscles are more numerous and are formed in the red marrow of bones. They live for 3 to 4 months and old corpuscles are destroyed in the liver. The corpuscles are biconcave with a thick rim. These contain a red pigment called Haemoglobin, which has an affinity for oxygen. It combines with oxygen to form an unstable

compound called oxyhaemoglobin. This gives up the oxygen in the tissues and becomes once again haemoglobin. So it helps respiration by transporting oxygen. The red corpuscles cannot

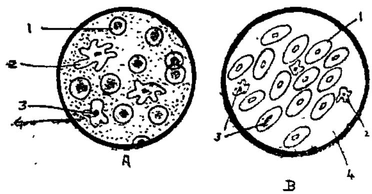


Fig. 168

A. Human blood   B. Frog's blood

1. Red corpuscle 2. White corpuscle 3. Nucleus 4. Plasma

move of their own accord and they usually group themselves as a pile of coins [The red corpuscles of frog, are nucleated and oval in shape. In Mammalian blood as the human blood, the nucleus is not seen and the cells are circular].

The white or colourless corpuscles are of different types. But they are mainly amoeba like with no permanent shape. They are smaller in number and move about with the help of pseudopodia. They devour foreign germs that enter the body and thus protect the body from diseases. So these corpuscles are called phagocytes. White corpuscles are classified into Basophils, Neutrophils, Eosinophils, Monocytes etc.

The platelets or thrombocytes are very small and they help to prevent loss of blood whenever there is an injury, by causing blood clot. The clotting of blood is a chain reaction brought about by an enzyme called Thrombokinase produced by the platelets.

Platelets ⟶ thrombokinase

↓

Calcium salt + Prothrombin

↓

Fibrinogen + Thrombin

↓

Fibrinthreads + Corpuscles = Clot

Functions of Blood:

1. Blood distributes the digested food.
2. Blood carries oxygen from the respiratory organs to all tissues.
3. Blood removes CO_2 from the tissues to the respiratory organs.
4. Blood takes the metabolic waste products to the excretory organs.
5. Blood provides all the raw materials required by the glands in the body.
6. Blood carries the hormones, which are the secretions of endocrine glands.
7. Blood distributes the heat evenly throughout the body.
8. Blood gives tissue pressure to the cells to counteract atmospheric pressure.
9. Blood keeps all the tissues moist and supple.
10. Blood prevents its own loss by clotting at the place of injury.
11. Blood protects the body from disease causing germs.
12. Blood carries antibodies to counteract toxins or poisons.

Germ Cells: The reproductive organ of an animal is called a gonad. The male gonad called the testis, produces the male gametes or sperms. The female gonad called the ovary, produces the female gametes or ova or egg cells.

The gametes are produced as result of reduction division or Meiosis. The production of gametes is known as gametogenesis. The gametes will have only half the number of chromo-

somes as the bodycells (i.e.) they will be haploid. During fertilisation 2 gametes (i.e.) haploid cells, meet and fuse to form a diploid cell or Zygote.

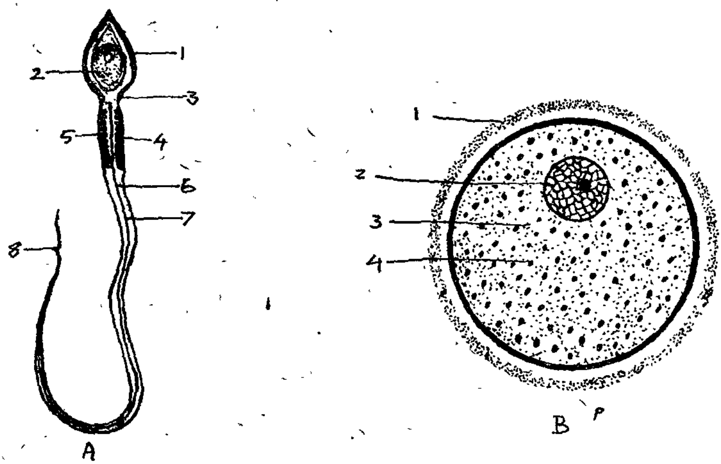


Fig. 169
Germ cells

A. Sperm
1. Head 2. Nucleus 3. Neck
4. Middle piece 5. Mitochondria
6. Tail 7. Axial filament
8. Tail (naked end)

B. Ovum
1. Vitelline membrane
2. Nucleus 3. Cytoplasm
4. Yolk granules

The formation of male cells or sperms is called Spermatogenesis. The sperm is very small. It has a head, a neck, a middle piece and a tail.

The head contains the nucleus, which occupies almost the entire head. There is a little cytoplasm at the tip called the acrosome. The neck contains a centriole. The middle piece contains a few mitochondria, which provide energy. The tail is long and is useful in swimming towards the female cell.

The ovum or egg cell is poduced by oogenesis. The egg cell is comparatively very big and it is usually spherical. The nucleus is not exactly at the centre. The end of the cell, towards which nucleus is seen, is called the animal pole. The opposite end is called the vegetal pole. The cytoplasm of the egg cell is full

of nutritive matter called the yolk. It appears as yolk granules (The amount of yolk may be little or may be heavy according to the nature of the egg). The egg cell is enclosed inside a membrane called vitelline membrane.

Many sperms surround an egg cell, but only one succeeds in entering it. As soon as the head of the sperm enters, the rest of the sperm is cut off. The egg cell develops a fertilisation membrane to prevent other sperms from entering.

The fertilised egg cell is now called a zygote. This divides repeatedly and rapidly to form numerous cells, which arrange themselves to become the young one.

CHAPTER XXV

Cytological Tools and Techniques, Microscopy

The study of cytology in modern times involves the application of various tools and techniques because the cell is a highly complex unit with different chemical, physiochemical and morphological aspects. The apparatus (tool) that has made possible the study of cells, which are normally invisible to the normal eye is called the Microscope. Particles that can be seen under the microscope are called microscopic particles:

Several microscopes are known today:

1. Light Microscope
2. Compound Microscope
3. Phase contrast Microscope
4. Interference Microscope
5. Polarizing Microscope
6. Flourescent Microscope
7. Dark field Microscope
8. U.V. (Ultra violet Microscope)
9. Electron Microscope

Several other techniques like birefringence, Dichorism, X-ray diffraction etc. are now used to describe the ultrastructure of cells.

PRINCIPLES IN MICROSCOPY

There are two main aims or principles in Microscope. They are:

1. The formation of a magnified (enlarged) image free from optical defects.

2. The achievement of contrast (i.e.) a difference in the intensity or colour between the object and the background.

A magnified image may be produced by increasing the resolving power of the lens. The resolving power may be defined as the property of an optical system to distinguish objects lying very close together (For example). In observing a double star, some individuals will be able to see only a single star, while others with better resolving power will be able to see two separate stars.

The resolving power may be calculated by considering an image formation in a convex lens. Consider a source of light P in front of a lens AB, only a very small portion of the light will pass through the centre of the lens, which will pass through it in a

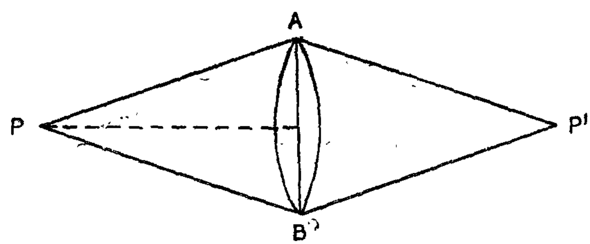


Fig. 170
Principle of a lens

straight line. The light rays that pass through the thinner edge of the glass, bend and are brought to a focus at P^1.

Now resolving power is given by the formula $r = k\lambda / NA$ Where k is a constant and is equal to $k = 0{\cdot}61$. Thus resolving

$$\text{power } r = \frac{0{\cdot}61 \times \lambda}{NA}$$

where NA is the Numerical Aperture.

If the $\angle APB$ is 2μ then the

NA=n sin μ where n is the refractive index of the medium. In this way the maximum resolution of visible white light has been found to be 0.25 (microns) (1 micron = 0.001 mm.)

A. Light Microscope

The light microscope is the most simplest of all microscopes. It consists of chiefly:

1. **The objective lens** which lies close to the object, and which has a high resolving power.

2. **Ocular or eyepiece** which lies nearer the observer (viewer), and which merely magnifies the object resolved by the objective.

The next most important part **is a light source** (usually sunlight) which is made to pass through the object by means of a **movable mirror adjustment,** which is a plano concave mirror. There is also a **mounted circular disc** on which the object can be placed and observed.

By means of the light microscope one can see in a magnified form only the external morphology of minute organisms or cells.

B. Compound Microscope

The essential parts of a compound microscope consist of (1) a light source (2) a condensing lens system to collect and enlarge further the image formed by the objective.

This consists of a body tube. At one end (lower end), is fitted the objective while the ocular or eye piece is seen at the upper end. In this microscope the magnification occurs in two stages. First an inverted and magnified image of the object is formed by the objective, and secondly this image is further magnified by the ocular or eyepiece. The maximum power of resolution of a good compound microscope is about 1500 times.

There are as many as three objectives of different magnifying powers attached to a revolving nose piece. A pair of focussing knobs for coarse and fine adjustment is present to facilitate the adjustment of the body tube for sharp focussing of the image. There is a mechanical stage attached to the general framework of the microscope, intended for holding a

glass slide on which the specimen to be studied is placed. The glass slide is kept in place by a pair of clips attached to the stage. The object is illuminated from below by reflected light from a plano concave mirror attached to the main framework beneath the stage. Between the reflector and the stage is a condenser and an iris diaphragm. The condenser can be raised or lowered by rack and pinion adjustment. This serves to control the intensity of light falling on the object. The entire system is mounted on a solid frame supported by a strong and heavy two pronged base.

A variation of this ordinary compound microscope is the binocular microscope which has two eye pieces permitting the viewer to observe the object with both the eyes.

(Refer Page 170 & 171 for Electron Microscope
Vide Chapter XVII.)

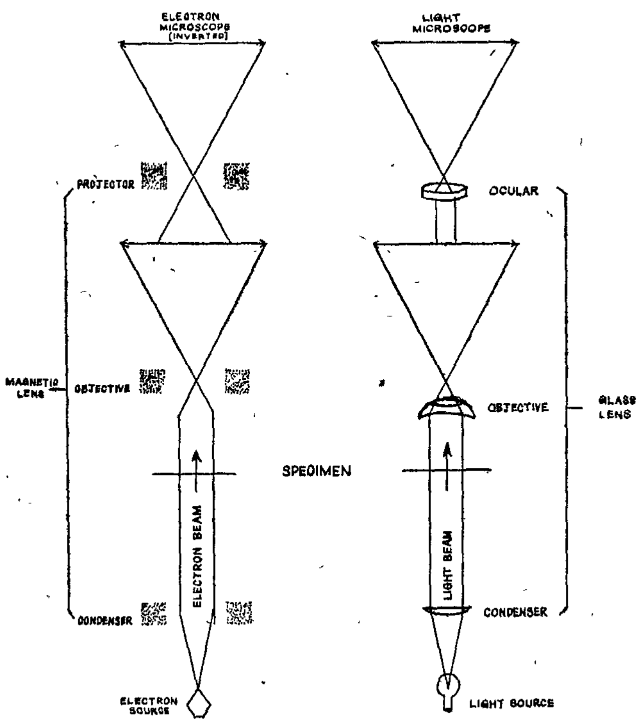


Fig. 171 Principles of Microscope

STAINING TECHNIQUES FOR LIVING AND FIXED CELLS

Vital dyes penetrate living cells and color certain structures without seriously injuring the cells. For example, neutral red may be used to stain the cytoplasm although in some cells it accumulates in vacuoles instead. Janus green B. stains mitochondria selectively. Methylene blue selectively stains the Golgi apparatus. Vital dyes are not entirely harmless, but they kill only after cells have been exposed to them for a long time. Sometimes the poisonous constituent (e.g. the heavy metals in Janus green B) may be removed from a vital dye solution making the dye much less toxic than the original sample. While helpful, the vital dye technique has only limited use because any of the cell organelles are not stained by such dyes and, in all cases, scattering of light obscures boundaries of structures. However dyes have been used to demonstrate that some cell organelles such as the mitochondria, Golgi apparatus and vacuoles are real structures present in the living cell.

Most of the early work on the nature of cell organelles was done with fixed and stained preparations. Fixing agents such as formalin, alcohol, acids, salts of heavy metals or mixtures of these, precipitate the proteins and render them insoluble. Next, water is removed from the fixed tissues by dehydrating agents, such as alcohol and the tissues are embedded in paraffin and sectioned with a microtome. The sections are affixed to slides and the paraffin is removed with xylol and washed in xylol- alcohol. By washing in decreasing concentrations of alcohol the sections are partially hydrated and the proteinaceous material of the cell is then differentially stained to distinguish the structures present. Natural dyes (e.g. hematoxylin) or basic anilin dyes. (e.g. safranin and basic fuchsin) stain the nucleus selectively, and acid dyes (e.g. orange G, eosin and fast green) stain the cytoplasm. The sections are then dehydrated with alcohol. To reduce scattering of light, it is necessary to replace the alcoholic medium with a substance having the same refractive index as that of the protein particles. This is accomplished with a clearing agent, such as xylol, which infiltrates among the protein particles. The preparation is then mounted in balsam, which has a refractive index about equal to that of the cell proteins. As a result, it is possible to look at the cell and see clearly the stained structures which are within.

CHAPTER XXVI

Genetics

Apart from identical twins, no two humans are exactly alike. This applies to all living things. That variety is the spice of life is not only true of human experience but constitutes a principle inherent in the very nature of Nature. At the same time, it is also a common observation that the distinguishing characteristics of a given species is maintained generation after generation. This is heredity. Genetics is the branch of biology concerned with the manner in which inherited differences and similarities come into being between similar organisms. Though it has been known for quite sometime that there must be underlying natural laws which are responsible for heredity and variation, it was only in the beginning of the 20th century that an adequate scientific foundation was available. It has been established from the vast amount of data gathered in studies of genetics, that all characteristics of any organism have hereditary as well as environmental components, although some traits are more immediately influenced than are others.

Methods of Genetic Study

Many techniques have been used by researchers in exploring the mechanisms of heredity and variation. The direct approach is experimental breeding; in human genetics study of twins, analyses and statistical procedures are more useful. Cytological investigations with regard to chromosome behaviour, biochemical and biophysical techniques to study the gene and its mechaniques are basic to the study of the genetics. In the laboratory, observations made on animals and plants have been most useful. Of these, the fruit fly (Drosophila melanogaster) is the experimenter's favourite, for it is possible to study 20 to 25 generations in a year. Biochemical and biophysical investigations have been done mostly on bacteria such as Escherichia coli, the common

colon bacillus and viruses. Electron microscopes, microspectrophotometers and autoradiography have been valuable tools in these studies.

Historical Background

Even in remote periods of history, men were making a conscious effort towards improved plant and animal breeding. Fantastic explanations of the mechanism of reproduction and sex determination were given. It was only with the development of the microscope in the latter part of the 17th century that it was possible to discover the details of sexual reproduction and reproductive mechanism. This laid the foundation of the discovery of genetic mechanism. Rapid progress was made on the sexual reproduction in plants and animals and it was Mendel (1822-1884) who was able to throw light on the problems of Heredity. He is therefore called the father of Genetics and though his findings have since then been modified, his conclusions constitute the foundations of the modern science of Genetics.

Mendel (1822-1884)

Father Gregor Mendel was an Austrian monk, who in his spare time experimented with plants in the Augustinian monastry garden at Brunn, of which he eventually became the abbot. After eight years of experiment and analysis Mendel presented his findings before the local Brunn scientific society in 1865 and published his results a year later. But the value of his work went completely unrecognized until 16 years after his death. In the year 1900, three diffeent scientists, Hugo de Vries of Holland, Karl Correns of Germany and Erich Von Tschermak-Seysenegg of Austria, almost simultaneously brought the importance of his work to the attention of the scientific world. Mendel entitled his paper simply as "Experiments in Plant Hybridization." He did not announce it for what it was, namely, the discovery of the age-old mystery of the laws governing the inheritance of traits.

Much of his success was due to the choice of material that he had selected for his investigations, viz., the garden pea or Pisum sativum which was an annual plant, with well defined characters and in which self fertilization was the rule. His good judgement in making crosses and studying the inheritance

of separate traits, rather than whole complexes of traits was another factor which gave him successful results. Further he maintained a record of his results with great precision, in which his knowledge of mathematics and statistics helped him a great deal. He studied seven pairs of characters in peas: (1) seed form (2) colour of seeds with coat (3) colour and seeds without coat (4) form of ripe pods (5) color of unripe pods (6) position of flowers and (7) length of stem.

Other geneticists who have contributed to our present knowledge in Genetics are Thomas Hunt Morgan (1866-1945), who received Darwin Medel in 1924 and in 1933 received Nobel Prize for his discoveries related to the hereditary functions of the Chromosomes, Sutton (1876–1916) Bateson and Punnet, H. J. Muller who received Nobel Prize for his discovery of the production of mutalions by means of X-ray irradiation, Beadle and Taturn who received Nobel Prize in 1958 for their discovery that genes act by regulating specific chemical process; Pauling, and a host of others.

LAWS OF MENDEL

Soon after entering the monastery, Mendel began trying to develop new colours in flowers. In doing so he acquired considerable experience in artificial fertilization. His interest was aroused by the surprising and unaccountable results that he sometimes obtained. Whenever he crossed certain species, the same hybrid forms cropped up with striking regularity. But when he crossed one of his hybrids with another, some very different characters sometimes appeared among their progeny. Others in his time had also noted this phenomena appearing with regularity. But Mendel, counted and figured the mathematical relationships of hybrid plants which was one of the main reasons for his success.

Mendel's experiment: Mendel planned his experiments with great care and foresight. He tried out enough plants to choose one, which had varied characters, each of which bred true to form, and which could be protected from foreign pollen during the flowering period. He found that the common pea was the

best to meet his specifications for an experimental plant. His next job was to make sure of his material. He obtained 34 distinct varieties of peas and planted them in his monastry garden. Except for one, each variety yielded offspring exactly like itself. He repeated the plantings during the second year. The results were the same.

Having made sure of the raw material, he selected 22 plants and from these, seeds with seven sharply contrasting pairs of characters. The following clear cut differences were selected by him

1. Form of ripe seeds—round or wrinkled:
2. Colour of the seeds-yellow or green.
3. Colour of seed coat-white or grey.
4. Form of ripe pods—inflated or constricted between the peas.
5. Colour of unripe pods - green or yellow.
6. Position of flowers–distributed along the stem or bunched at the top-axial or terminal.
7. Length of the stem - tall (6 or 7 feet) or dwarf (3/4 to 1-1/2') .

First step in experiment

Each kind of seed was collected and in spring he planted them in separate plots. When they were ready to bloom, he opened the buds of 'wrinkled' plants, pinched off the stamens to prevent the peas from fertilizing themselves, and tied a little paper bag around each one to protect the exposed stigma. As soon as the pollen had ripened in the 'round' pea plot he collect ed the pollen and dusted it on the stigma of the 'wrinkled' pea plants. The flower was protected by a bag, from bees which may bring pollen from other flowers. To make certain that his experiments were not affected by, which plant served as the seed present, Mendel also reversed his fertilization procedure. Some of the pollen from the wrinkled peas was deposited on the prepared stigma of the round. Similarly all the other character-bearing plants were treated. Altogether he made 287 cross fertilizations on 70 plants.

Observation

The experiment which gave him the first result was the one in which he had chosen the colour of unripe pods-green or yellow. He found that, as the pods appeared, all of them were green, whether they came from parents that produced yellow pods or sprang from parents with green pods. That is, yellow and green parents alike had poduced green offspring. Confirmation of this striking result came as the other characters he had chosen exhibited themselves in the next generation. In all the seven contrasting characters he had chosen he found that, in each pair of contrasting characters, only one of them was evident in the next generation. He designated the hereditary trait that prevailed as a **dominant** and the other trait that disappeared in the first generation (F1) or first filial generation as **recessive.**

Second step in experiment

Mendel's next step was to collect the first generation seeds or **hybrids** and to cross it with another hybrid. He did not operate on the buds but permitted the peas to fertilize themselves in the natural way and thus obtained a cross of two identical hybrids.

Observation

He found that the trait that had disappeared in F1 reappeared in F2 (Second filial generation). It meant that, that trait had not been lost but this heritage from the grand parents persisted and had come forth again. He counted the plants which showed the traits and found that in all cases the ratio of one trait of a contrasting pair to another was nearly 3: 1. From 258 plants of seed colour he obtained 8023 peas. Exactly 6022 were yellow and 2001 green. Of 253 hybrid plants of the seed form, experiment produced 7324 peas, of which 8,474 were round and 1850 wrinkled. Thus the average of the entire group was three to one.

Third step in experiment

Mendel continued his plantings through six or seven generations in all cases. As he did so, he learned that the 3 to 1 ratio, which was based on the appearance of peas, was actually 1:2:1 ratio when the true nature of the peas was revealed by later plantings.

Mendel planted the wrinkled peas from the first hybrids in a separate plot. They yielded only wrinkled peas and continued to produce only wrinkled as long as he planted them and permitted them to fertilize themselves. They were pure recessives. But the round peas from hybrid parents produced both round and wrinkled seeds. One fourth were true rounds and as long as they were planted, would yield only round peas. Two fourths were round in appearance, but were actually hybrids and would produce round and wrinkled peas in a steady 3 to 1 ratio. The wrinkled peas from the union were again pure wrinkled and never yielded anything but their own kind.

Thus it was clear that appearance meant little or nothing. The surface differences of offspring were great and yet below the surface lay still other differences and possibilities for variation.

Interpretation

With the clarity and simplicity of a genius, Mendel labelled the 'dominant' in the union with a Capital A, and the recessive with a small a. A constant dominant thus would be formed by the coming together of two A's, and it would be described as AA; a hybrid by either Aa or aA; and a recessive by aa. With this understanding it was possible for him to chart this coming together and separation and the clearcut results that it produced. He had no way to look into the egg and pollen cells to search for the factors responsible for this hereditary pattern. But he reasoned out the biological basis that had to underlie them. He formulated three laws of inheritance from the above experiments.

1. All living things are a complex of a large number of independent heritable units–**Law of unit characters**.

2. When each parent contributes the same kind of factor and the two come together in the offspring, a constant character is produced. But if one parent contributes one kind of factor, say A, and the other another, say a, a hybrid results. When the hybrid forms reproductive cells, the two differentiating elements segregate themselves again and thus are free to form new combinations in the next union–**Law of segregation**.

3. The factors are unaffected by their long association in the individual. They emerge from the union as distinct as they entered it. This was Mendel's ultimately famous **law of the purity of gamates.**

The law of segregation is generally known as the first Law of Mendel and his experiments wih one pair of contrasting characters as Monohybrid cross and the pattern of inheritance as Monohybrid inheritance. The 3:1 ratio is called Monohybrid ratio.

Mechanism of Monohybrid Inheritance

Mendel's experiment on one pair of characters viz. wrinkled and round peas may be graphically illustrated as follows:

Parent: Round × Wrinkled
F-1 all were round (hybrid) } —1st step in experiment

F-1 Round hybrid × Round hybrid } —2nd step in experiment

F-2	Round	Round	Round	Wrinkled	(3:1)
	↓	↓	↓	↓	
F-3	Round	Round & wrinkled	Round & wrinkled	wrinkled only	

Interpreting the above using the principles of inheritance of Mendel, the monohybrid inheritence may be illustrated.

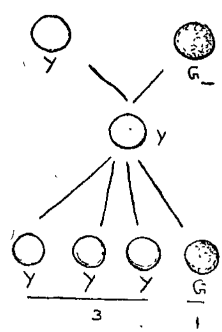


Fig. 172 Monohybrid ratio
Y. Yellow G. Green

Let us assume that in each gamete there is a factor e.g. R or r (now known as gene) present which is capable of determining a character in the offspring. In the zygote these factors are brought together at fertilisation (RR or rr) when the factors are identical as above they are called homozygous (RR) when they are different they are called heterozygous (Rr). A pair of contrasting characters are called allelic pairs of allelomorphic pairs. When a male with genic composition of RR is crossed with a female with genic composition rr or vice versa, the gametes of the parents, each with a single factor (due to meiotic division) unites with the gamete of the other sex (Rr) and forms a zygote. Since one of the contrasting characters is dominant and the other is recessive, they have round seed coats.

When the hybrid seeds (Rr) are planted and allowed to self fertilise, each hybrid produces two types of gametes, namely, R and r. Four combinations are possible RR, Rr, rR and rr. When they are observed, 3 of them (RR, Rr, rR) appear alike, since they possess the dominant factor R and have round seed coats while one of them rr shows wrinkled seed coat. Thus 3:1 ratio is arrived at. This is called phenotypical ratio. But when their genetic make up is examined, this ratio is modified as 1:2:1 ratio (genotypic ratio), RR and rr being pure and Rr and rR being hybrids, which in turn will split into 1:2:1 ratio again if self fertilised.

In order to verify his theory that hereditory factors remained distinct and issued unadulterated out of the hybridized pair, he

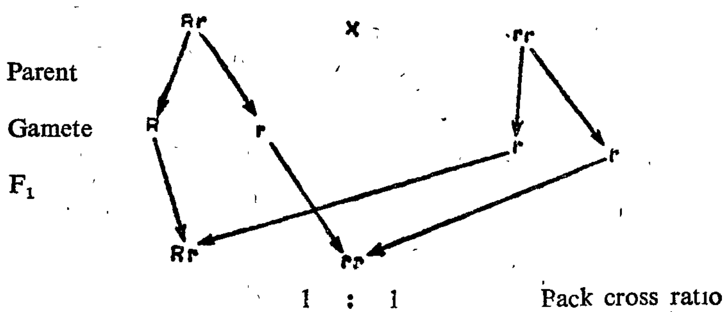


Fig. 173

performed a **test cross** or **back cross.** It is also a test to find the purity of an individual, whether it is pure strain or hybrid.

In this back cross, the hybrid (Rr) is crossed with the recessive parent (rr). He worked out the combinations that would result.

These results were obtained as predicted. Thus the most severe tests had been applied and Mendel's theory had been proved with a certainty that exceeded all expectations. Many such experiments have been performed since Mendel's time on Drosophila and more than 300 generations of this insect have been reared without contamination. The method of back cross has important practical applications in plant and animal breeding, and in producing disease resisting varieties of plants and animals. For example, a particular type of tomato is of good quality but is not resistant to fungal diseases; wheareas there is another variety which is small in size but is resistant to wilt. This is due to a dominant gene I. These two varieties II and ii are crossed and the hybrid Ii is again backcrossed with the recessive parent ii Of the resulting plants Ii, and ii the heterozygous, immune plants are saved and the recessive ones discarded. By further breeding experiments, a pure breeding immune plant can be obtained.

Modifications of Mendel's Law of segregation

Since Mendels time, science has advanced a great deal and our knowledge regarding the laws of inheritance has increased. Subsequent breeding experiments with a variety of organisms showed that the pinciples laid down by Mendel were not universally applicable.

Mendel's principle of Law of unit characters did not apply to all cass, but there were several cases where one gene was not responsible for one character but a trait involved the combined action and interaction of many genes. Similary, exception to Mendels law of segregation were met with when the chromosomes failed to segregate and cases of non disjunction were met with. There were also case of incomplete dominance in several cases, as a result of which, the hybrid of the F1 generation showed intermediate characteristics, the parental traits having blended in them. Classical example of incomplete dominance is seen in the 4'o clock flower or Mirabilis jalapa, as shown by Correns. The flowers of this plant are red or white. Both types breed true. If the two types of plants are crossed, the F1 generation gives pink flowers showing incomplete dominance. If the hybrids

are interbred red, pink and white flowered plants are obtained in the ratio of 1:2:1. The genes which thus express themselves in a partial and intermediate manner are called intermediate genes. Similar cases have been met with also in experiments with Andalusian fowls.

Though the expression of the genes is partial, there is no actual blending of the genes neither is there any contamination of genes. When the pink flowered hybrid 4'O clock plant is backcrossed with a recessive homzygous white flowered plant 1:1 ratio of pink and white flowers are obtained thus confirming that the pink flowered plants are hybrids, with heterozygous genes.

Cases of incomplete recessive genes have also been met with. In a hybrid for example, the recessive gene has small effect when heterozygous.

Modern Evaluation of Mendel's conclusions

1. Mendel considered a single gene to be responsible for a single trait. It is now known that many genes, perhaps all the genes present in an organism have something to do with each trait. Certain differential genes however are known to influence basic reactions and thus to be responsible for alternative end poducts or phenotype.

2. It is the genes that are inherited, not the traits. Genes behave as separate units whereas traits usually result from complex interactions involving many genes.

3. Dominance is not an inherited property of the genes but is influenced by factors in the external and internal and genetic environment. Dominance may be explained on the basis of modifiers or genes other than the one directly concerned that are present in the genetic environment.

4. Genes are in chromosomes and groups of genes sometimes behave as units.

DIHYBRID INHERITANCE

Being an excellent mathematician Mendel was quick to note and pursue the revealing indication of order in heredity. He realised from the results obtained, that he was simply obtaining every combination that could be formed by the separate factors present. If A and a were combined, only one combination could be *formed:* Aa. If Aa and Aa came together, four combinations could be made: AA, Aa, aA, aa and this was exactly what happened.

If two series were united—A+2Aa+a and B+2Bb+b, 16 different groupings could be produced. And as the dominants would determine the appearance of each group, the result would be 9AB, 3Ab, 3aB and 1ab as exactly what he found in the pods when he took two pairs of contrasting characters into consideration.

Experiment:

1st step: Mendel crossed a variety of pea plant showing two pairs of contrasting characters-wrinkled seed, green in colour; and Round seed, yellow in colour.

Observation: The hybrid offspring were all round and yellow. These are dihybrids.

2nd step: The dihybrids were inter bred.

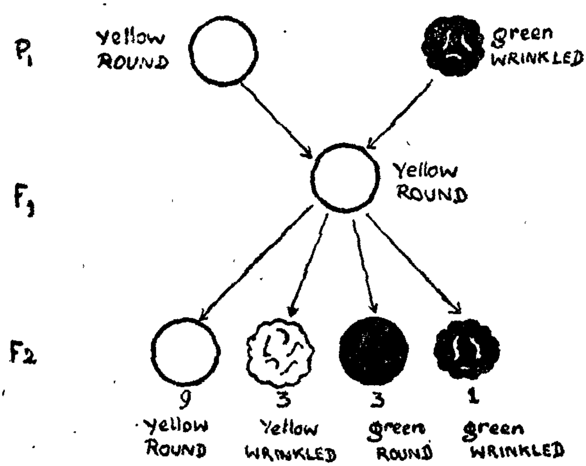


Fig. 174 Dihybrid ratio

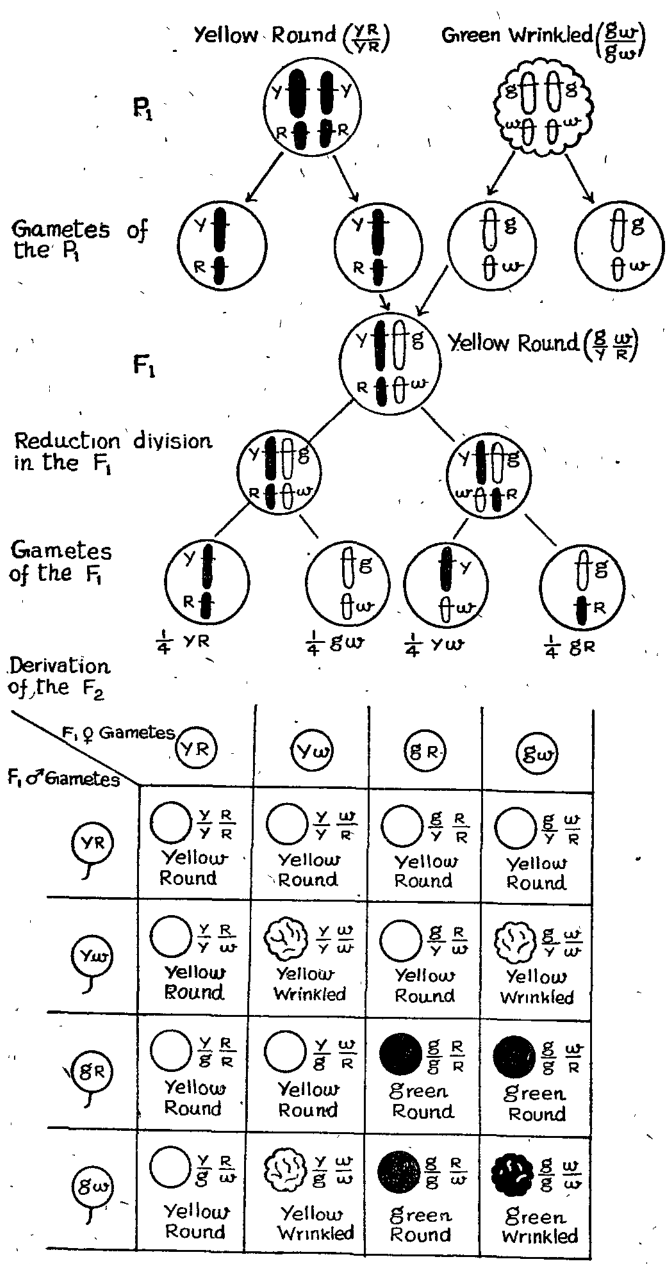


Fig. 175 Dihybrid Ratio Table

Observation: The F2 generation were in the ratio of 9:3:3:1 his actual results were, 315 Round and yellow, 101 wrinkled and yellow, 108 Round and green and 32 wrinkled and green.

Interpretation

The F1 generation showed that the gene for round seed coat and yellow colour were dominant over the wrinkled seed coat and green colour. Let us assume that here is a separate gene for each character and that the genes occur in pairs in the cells of the plant. The two parent generations will have then a genic composition of RRYY (round and yellow) and rryy (wrinkled and green). Each gamete receives only on member of each pair of genes and, so, the gametes formed by RRYY will be RY and gametes formed by rryy will be ry. When they fuse, a zygote is formed of genic make up RrYy which is a dihybrid, expressing the dominant traits R and Y (Round and yellow). This hybrid can produce 4 gametes-namely RY, rY, yR, and yr. When two such hybrids are crossed, all the 4 kinds of male gametes have an equal opportunity of fertilising the 4 kinds of eggs. 16 possible zygotes will be formed which can be shown diagrammatically by checker board method introduced by the British geneticist R.C. Punnet.

Inference

By this experiment and others similar to, using trihybrids, tetrahybrids etc. Mendel framed another law, called Law of **independent assortment** or the law of free recombination. It states that the pairs of alleles conditioning the different pairs of characters in the offspring are distributed independently of each other in the way they recombine in the formation of characters. New combinations as well as old ones of genes and traits will occur and it will be seen that every trait is inherited independently of every other trait.

It is further obvious that each gene behaves exactly as it does in a monohybrid cross. If, in the dihybrid inheritance, one pair of characters alone are taken into consideration, Mendel's result shows 3/4 yellow and 1/4 green (480.140) and 3/4 round and 1/4 wrinkled (423:123). This shows that the dihybrid inheritance pattern is not anything new but that it is merely a

combination of monohybrids, each of which behaves just as it does when studied separately.

Dihybrid backcross

To prove that the hereditory factors separated and recombined as indicated he performed test cross with double dominants and double recessives. A dihybrid AaBb is crossed with AABB. The hybrid produces 4 kinds of gametes–AB, Ab, aB ab where as the other produces only one type of gamete, *viz.*,

	AB	Ab	aB	ab
AB	AABB	AABb	AaBB	AaBb

AB: As each pair would contain a dominant, all should have round yellow seeds.

If the test cross is made with double recessive parent, the result in different.

	AB	Ab	aB	ab
ab	AaBb	Aabb	aaBb	aabb
	Round	Round	wrinkled	wrinkled
	yellow	green	yellow	green

All the 4 types appear in equal numbers. In all his experiments, there appeared all the forms which the proposed theory demanded.

Modifications of Mendel's law of Dihybrid inheritence

As with Mendel's first set of principles subsequent work showed that Mendel's dihybrid ratio of 9:3:3:1 was not always achieved, due to lack of dominance in independent resortment interaction of genes and due to the effects of linkage of genes. Further it has been shown that a single gene is capable of exerting influence upon more than one character (pleiotropy).

The dramatic triple discovery of the work of Mendel by a Dutch, a German and an Austrian scientist and their simultaneous confirmation of his brilliant findings caught world-wide attention. The greatness of Mendel was recognized and one of the oldest desires of man, to know how the distinctiveness and the very form of living things are passed along from parents to progeny was at long last satisfied.

CHAPTER XXVII

Gene Action

Gene is a section of a polydeoxy ribouncleotide chain, divisible both by mutation and recombination and concerned with a specific unitary function.

In Mendel's days and soon after, the interpretations of gene structure and function were limited in scope and understanding. Answers to questions regarding gene action were sought in the relationship between genes and specific biochemical actions, when it was discovered in the early 1900's by Sir Archibald Garrod that a number of congenital metabolic diseases in man were caused by human biochemical deficiencies involving enzymatic abnormalities. These suggested a relationship between enzymes and genes, but had very little influence on contemporary genetic or biochemical thought. In the 1930's George Beadle, Brosi Eprussi and Edward Tatum began a series of experiments on Drosophila to study the eye-pigment formation. They were able to show that the synthesis of normal eye pigment proceeded through a series of chemically identifiable intermediates and that each step was under genetic control. Beadle and Tatum formulated the "one gene, one enzyme" concept in the early 1940's, which states that the gene exerts its influence on the phenotype through its role in the production of an enzyme.

The pink bread mould **Neurospora** has proved to be very useful for experiments on chemical genetics. Neurospora grows along the surface medium like stale bread or can be grown in the lab on agar jelly supplied with sugar and biotin. Apart from sugar as an energy supply, the only molecule Neurospora needs in ready made supply is the vitamin Biotin. Bll other molecules involved in its biochemical system are made by the mould itself. The cell is effecting a meshwork of chemical synthesis. According to one-gene-one enzyme hypothesis, an

enzyme (b) which is necessary to transform molecule A to molecule B, is controlled by a paricular gene. If so, a mutation (or a sudden change) that may take place in the gene, will fail to produce the necessary enzyme (b) and the reaction A-B will also not take place. This was found to be the case in Neurospora, in which mutation of a gene was brought about by irradiation. They were allowed to develop further and their spores were grown in a complete medium with vitamins, aminoacids etc. From this culture, transfers of asexual spores were made to minimal medium. It was observed that the mutant was unable to grow in it due to the gene mutation. The growth deficiency is assigned to the locus of some gene. Some single substance added to the minimal medium satisfied the growth requirements. This suggests that such substances represent metabolites in Neurospora whose bio-synthesis has been blocked in the mutant strain.

Studies of this kind have been carried out with many organisms, particularly among the bacteria, fungi and algae. Mostly these variants are characterized by requirements for particular vitamins, amino acids or other essential growth substances.

Research in the last two decades has verified and extended this basic conclusion of Beadle and Tatum. But some instances have been found where single gene mutations may give rise to multiple growth factor requirements, and also cases where more than one gene is involved in the control of the activity of a single enzyme. For example, in man two different genes are known to control the structure of the haemoglobin molecule. However study of the haemoglobin from mutant individuals reveals that in fact the different genes control the structure of the different polypeptide chains of the molecules. These findings suggest that one gene-one enzyme hypothesis might be revised to one gene-one polypeptide hypothesis.

It must be pointed out here that although most of the studies of gene action are concerned with the control of enzymes, it should not be thought that all genes act by controlling the structure of polypetide chains of enzymes. Many genes produce proteins whose cellular function is not enzymatic. For example,

the virus genes determine the structure of the polypeptide chains of enzymes that make up the coat of the virus.

Gene Action in Man

The English physician, Sir Archibald E. Garrod was the first to point out certain physiological abnormalities in man and he put forward a theory that some of these abnormalities were due to absence of enzymes which were present in normal persons. In mutants, the enzyme was not produced, and so the disease was brought about. This was confirmed much later.

One such biochemical disorder is phenylketonuria of PKU brought about due to defective metabolism of an aminoacid, phenylalanine. There are three pathways by which phenylalanine is metabolised, each step being catalysed by an enzyme. Mutations may interfere with the production of a particular enzyme, as a result of which phenylalanine is not metabolised due to the blocking of one particular pathway. Then the phenylalanine accumulates in abnormal amounts and the excess diffuses into the blood and some of it will be excreted in the urine where it can be detected by simple chemical tests. When another pathway is blocked it causes genetic goitrous cretinism and when the third pathway is blocked it causes another disease called alkaptonuria. Thus a defect in the different pathways of the breakdown of a single acid can produce abnormalities. It is possible that similar defects exist in the metabolism of other amino acids.

Another disease called sickle cell anemia is brought about due to the replacement of one aminoacid by another at one particular point in the chain of aminoacids forming the haemoglobin.

F. Jacob and J. Monod have tried to form a plausible theory with regard to the possible type of control of the gene action. As a result of experiments on E. Coli, they have formulated a model, according to which there are three kinds of genes.

1. **Structural genes** which produce mRNA, which governs protein production by ribosomes.

2. **Operator genes** which act as switches to stimulate the synthesis of RNA by the structural genes. A single operon may govern the activities of a cluster of structural genes, perhaps through histone.

3. **Regulator genes** which produce substances which act on the operons to turn them off so that they do not stimulate the structural genes. Regulator genes may be sensitive to external factors such as hormones, organizers etc.

INTERACTION OF GENES

In the succeeding chapters it is pointed out that the different pairs of contrasting factors (alleles) may be independent of each other in their segregation and recombination patterns. Independence of gene transmission however does not necessarily imply independence of gene action. In fact, no gene acts by itself and its final expression is the phenotype of the individual, for an individual is the result of many integrated reactions. Evidence of this interaction can be demonstrated by considering modifications of the dihybrid phenotypic ratio of 9:3:3:1. Modifications of this ratio are of two kinds.

1. those in which there are more than 4 phenotypes

2. those in which certain tvpes cannot be distinguished, so that there are fewer than 4 phenotypes.

More than 4 phenotypes

1. The coat colour in cattle is red and white. A cross between animals having these coat colours however has roan colour due to incomplete dominance of red colour. Another pair of alleles, for horned condition and hornless condition may be considered in conjunction with coat colour.

Let us consider a cross between animals of horned-white and hornless–red. Let us assume that the alleles for horned and hornless condition is represented by genes h and H (Hornless is dominant) and coat colour represented by genes R and r.

Then,

P....Hornless Red × Horned White

F_1 Roan and Hornless × Roan and Hornless

F_2 6:3:3:2:1:1

PARENT: *RRHH* × *rrhh*
(RED, HORNLESS) (WHITE, HORNED)

F1 *RrHh* × *RrHh*
(HORNLESS, ROAN) (HORNLESS, ROAN)

GAMETES: *RH*, *Rh*, *rH*, *rh*, *RH*, *Rh*, *rh*, *Hr*,

	RH	*Rh*	*rH*	*rh*
RH	*RRHH* HORNLESS RED	*RRHh* HORNLESS RED	*RrHH* HORNLESS ROAN	*RrHh* HORNLESS ROAN
Rh	*RRHh* HORNLESS RED	*RRhh* HORNED RED	*RrHh* HORNLESS ROAN	*Rrhh* HORNED ROAN
rH	*RrHH* HORNLESS ROAN	*RrHh* HORNLESS ROAN	*rrHH* HORNLESS WHITE	*rrHh* HORNLESS WHITE
rh	*RrHh* HORNLESS ROAN	*Rrhh* HORNED ROAN	*rrHh* HORNLESS WHITE	*rrhh* HORNED WHITE

Fig. 176

This ratio is obtained due to the fact that there are three possible coat colours and two possible stalls of horns.

The above can be illustrated using the symbols for genes and checker board, thus:

R: Red Coat

r: White coat

H: Hornless condition

h: Horned condition

$\frac{6}{16}$ Hornless Roan

$\frac{3}{16}$ Hornless Red

$\frac{3}{16}$ Hornless White

$\frac{2}{16}$ Horned Roan

$\frac{1}{16}$ Horned Red

$\frac{1}{16}$ Horned white

N.B: In one pair of alleles, H and h, one is completely dominant over the other; whereas in the other pair, R and r one is incompletely dominant over the other.

2. In the flower colour and types of leaves of Snap dragon flower, in both pairs of alleles there is lack of dominance. Thus when red and white flowered plants are crossed, in F1 generation, the flowers are pink; when plants with broad and narrow leaves are crossed, in F1 generation the leaves are inter mediate. In such an instance, when a cross is made between red flowered and broad leaved plant, and a plant with white flowers and narrow leaves, the F1 generation has pink flowers and intermediately shaped leaves. When the hybrids are interbred, the resultant ratio is 1:2:2:4:1:2:1:2:1. This can be worked out by the checker board method by using the following symbols R and r for colour; B and b for leaf shape.

RR: Red

rr: White

Rr: pink

BB: Broad leaves

bb: Narrow leaves

Bb: intermediate shape

Less than 4 phenotypes

When one trait depends not on a single pair of genes but more than one pair, then the number of phenotypes is lessened.

In the coat colour of mice, the typical wild condition is called agouti. Some mice however are black in colour and there are others with white colour (albino) When an agouti is mated with an albino, the F1 generation are all agouti, which when interbred give 9/16 agouti, 3/16 black and 4/16 Albino.

This occurrence is explained as follows: The gene C is thought to determine the production of an enzyme which by oxidizing chromogen produces colour. The particular colour produced will however depend on other genes for spcefic colours. So, the coat colour, which is **one** trait, is determined not by one pair of alleles (as in monohybrid inheritance) but by two pairs of

alleles C, c (production of enzymes or absence of enzyme necessary) to produce colour and R, r (for the actual colour of the coat) All coloured rats. (Whatever may be the colour) must contain at least one of the dominant gene (C) for colour. In albinos however, the alleles for colour are homozygous and recessive (cc).

Thus: RRCC: Agouti
rrcc: Albino (white)
RrCc: Black

Using these symbolism checker board can be worked out. The modification of 9:3:3:1 ratio here, is due to the fact that two classes of animals, which are normally different phenotypically cannot be told apart; the one with cc (homozygous, recessive for colour) is an albino, whether it contains Rr or rr. Thus one pair of alleles (cc) masks the expression of another pair of allele (Rr or RR) . In other words, the gene c is said to be **epistastic** to R and r. Since the epistastic gene c is recessive to C this is called **Recessive epistasis.**

Dominant epistasis is met with in the coat colour of dogs. B and b are the alleles for coat colour and I and i are alleles which control the production of pigment; I prevents the development of pigment, even though specific colour genes may be present.

Thus: BBII: White
bbii: brown
BbIi: White

The 9:3:3:1 ratio here is modified to 12:3:1

Both dominant and recessive epistasis occur in some cases. For instance, Leghorns, one variety of hens, are white because of dominant gene I which inhibits the colour, even though C, a dominant gene for colour may be present it cannot be expressed if I is present. Another variety of hens, called white silkie white is also white, but this is due to a recessive gene c (colour gene); so if cc is present, even though they carry colour genes, they cannot be expressed in the absence of chromogene.

Thus: IICC: White leghorns.
iicc: White silkie
IiCc: White

The modified ratio obtained in this cross is 13:3 of which 13 are phenotypically white and 3 are coloured, for, iiCc or CCii will be coloured.

When each of the two pairs of alleles contains a recessive epistatic gene, it is called **duplicate recessive epistasis** and the modified ratio is 9:7 P, p, R, r may be taken as two pairs of alleles, acting on flower colour in such a way that when both P and R are present, the flower has purple centres and all other combinations of these genes produce yellow centres.

Thus: PPrr: yellow centre
ppRR: yellow centre
PPRR: } Purple centres
PpRr: }

When plants PPrr and ppRR are crossed the purple hybrids Pp Rr are produced. When interbred they give 9/16 purple and 7/16 yellow centred flowers. Here P is epistatic to R and r; in addition r is epistatic to P and p.

Human deaf-mutism follows this pattern of inheritance.

Duplicate dominant epistasis occurs when each of the two pairs of alleles contain a dominant epistatic gene. In this case, presence of a single dominant gene is enough to produce feathered shanks in fowls.

Thus FFSS: feathered shank
ffss: unfeathered
FfSs: feathered.

The modified ratio here is 15:1.

Duplicate gene interaction is met with in pigs where sandy colour is determined by two pairs of alleles R and S.

RRss: sandy colour
rrSS: sandy colour
RrSs: red

The modified ratio is 9:6:1.

CHAPTER XXVIII

Multiple Alleles & Blood Groups

(A. B. O. Rh. Systems)

The genes used in the previous section have been presented as each possessing a single allele; however there are cases where more than a pair of alleles, which occupy the same locus on the chromosome may control a trait. All genes which occupy the same locus on a chromosome are called multiple alleles. An example of multiple allele inheritance is the blood groups of man. Three genes are responsible for the inheritance of blood groups.

The first serious medical attempts at replacing human blood of victims who had suffered serious blood loss were unsuccessful and evoked severe reactions and often death. In 1900, Karl Landsteiner discovered the explanation for these adverse results, as due to an effect of immune response.

Each kind of micro organism, contains a wide range of macromolecules which act as antigens. If and when a particular antigen gets into the body, it stimulates certain cells, derived from lymphocytes, to produce a corresponding protein called an antibody. The antibody combines with and in some way neutralizes the antigen, destroying the micro organism in the process. This can take place in several different ways, but generally the antibodies either stick to the surface of the micro organism so making them clump together (agglutination) or they may cause them to burst (lyse). The production of antibodies in response to antigen is called the **immune response.**

In man, any living material, introduced into the body is treated by the recipient as 'foreign' and antibodies react against it. When the blood of one person is transferred to another, if the blood of the two individuals is *not compatible*, the donor's RBC's clump together in groups (agglutination) which may result in blockage of the recipient's blood vessels. This is because antigenic

substances called agglutinogens are present on the surface of red blood cells, which are complementary to antibodies present in the recipient's plasma. Unlike normal immune responses, the recipient does not actually produce antibodies in response to the donor's blood. They are present all the time, agglutination occuring if the donor's blood happens to contain the corresponding antigens. Each individual usually has antibodies against those red cell antigens that he lacks. Why? The answer is not entirely certain. Perhaps we are all exposed to substances (in our food, from injection or from bacteria living in our intestine) that have antigenic determinants similar to A and B. We would proceed to synthesize antibodies against them if they did not resemble 'self' but not if they did. Certainly materials carrying A-like or B-like antigenic determinants turn up from time to time (e.g.) as contaminants in vaccines.

ABO System

The antigens or agglutinogens on the red blood cells are called the A and B antigens, and the blood types have been classified into A group (having antigen A), B group (having antigen B) AB group (having both antigens A and B) and O group (having neither).

The basic principle to be observed is that the blood introduced into the patients body must not contain RBCs which can clump. It is not serious if the introduced blood contains antibodies against his own RBC's because the antibodies will quickly be diluted by his own plasma. Hence type O blood has been called the **'universal donor'** because O red cells cannot be clumped and the antibodies will be quickly diluted by the recipient's plasma. Similarly AB blood is called the **Universal recipient** because it contains no antibodies to clump RBCs introduced into it. (Table 1 and 2 given below)

TABLE-1

Summary of Reactions that occur when Bloods of Different Groups are mixed

		Oab	**Ab**	**Ba**	**ABO**
	Oab	—	—	—	—
	Ab	+	—	+	—
Donor	Ba	+	+	—	—
	ABO	+	+	+	—

Key:-

+ = agglutination
— = No agglutination
Capital letter = antigens in corpuscle
Small letter = antibodies in plasma

TABLE–2
ABO System

Blood group	Antigens in RBC	Antibodies in Plasma	Can donate to	Can receive from
A	A	Anti B (b)	A, AB	O, A
B	B	Anti A (a)	B, AB	O, B
AB	AB	Neither	AB	O, A, B AB
O	O	Anti A & Anti B (ab)	O, A, B AB	O

In actual practice doctors prefer to match blood types exactly when carrying out blood transfusions.

AGGLUTINATION TESTS FOR BLOOD GROUPS

On a glass slide, is placed a drop of type A serum (containing 'b' antibody) and a separate drop of type B serum (containing 'a'

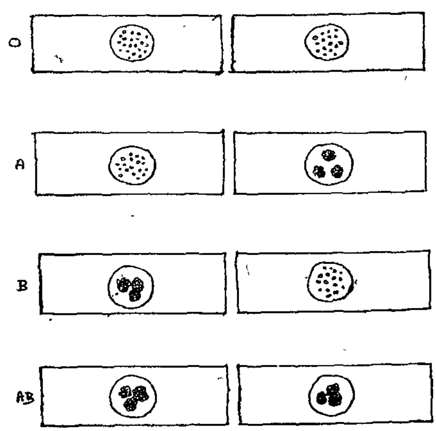


Fig. 177 Addition of Blood Type
Type A Serum Type B Serum

antibody). When a drop of type O blood is added to each drop (O blood has neither A or B antigens) there is no agglutination of red cells in either drop. If a drop of type B blood is added-agglutination occurs with type A serum; type A red cells are agglutinated by type B serum and type AB cells are agglutinated by both sera.

GENETICS OF ABO TYPE

The Landsteiner blood types are inherited and its genetics is well understood. The type is controlled by multiple alleles. An allele is the alternate form of a gene that may occur at given gene locus, one inherited from each parent. The term multiple allele is applied to the three or more genes that can occupy a single locus.

There are 3 alleles concerned in the genetics of ABO system; these are I^A, I^B and i. Gene I^A provides the code for the synthesis of a specific protein, agglutinogen A, in the red cell. Gene I^B leads to the production of a different protein, agglutinogen B. Gene i produces no agglutinogen. Neither gene I^A or I^B is dominant to the other. The symbols I^A, I^B and i are used to emphasize that all three are alleles at the same locus. Individuals with geno types I^A I^A and I^A i make up blood group A. Those with geno types I^B I^B or I^Bi comprise blood group B. Individuals with blood group O have the gene type ii. When both the I^A and I^B genes are present in the same individual both agglutinogens A and B are produced in his red cells and the individual belongs to blood group AB.

Blood group	Genotypes
A	I^A I^A or I^A i
B	I^B I^B or I^B i
AB	I^A I^B
O	ii

Applications

These blood types are genetically determined and do not change during a person's life time. Determining the blood types of the individuals involved, may be helpful in settling cases o disputed parentage (Table). Such blood tests can never prove that a certain man is the father of a certain child, but only whether or not he could be its father. They may definitely prove that he could not be father of a certain child.

EXCLUSION OF PATERNITY BASED ON ANALYSES OF BLOOD GROUPS

Child	Mother	Father must be of type	Father cannot be of type
O	O	O,A,B	AB
O	A	O,A,B	AB
O	B	O,A,B	AB
A	O	A, AB	O,B
A	A	A,B,AB,O	—
B	B	A,B,AB,O	—
A	B	A,AB	O,B
B	A	B,AB	O,A
B	O	B,AB	O,A
AB	A	B, AB	O,A
AB	B	A,AB	O,B
AB	AB	A,B,AB	O

With the development of techniques for determining the blood types of mummies and even of skeletons, the use of blood tests in anthropology has been considerably broadened.

DISTRIBUTION OF BLOOD TYPES

Some notable difference have been found in the relative frequency of the different types in different races. The proportion of blood types in a population remains constant from one generation to the next as long as there is no inter marriages with other groups.

In the ABO blood type system, O is the most common type; it is found in over 50% of individuals of most populations. Type B is almost completely absent in American Indians and Australian aborigines, but becomes increasingly common in Europe, Africa, India and Asia. With its high frequency in Asia, type B is often called the 'Asian gene' and some people believe that it was distributed from Asia to Europe by the successive waves of invasion by Mongols. Blood type A is actually of two types- A1 & A2. A1 occurs in American Indians, Asians and Pacific

islanders and Australian aborigines while A2 is limited almost completely to Europeans and their descendants.

To summarise, B & A2 are almost completely missing in American Indians, B is low and A2 high in Europeans, B is high and A2 is rare in Asians while Australian aborigines, American Indians and Polynesians all have no A2 and little B.

TRANSFUSION OF BLOOD

The transfusion of whole blood, plasma or plasma fractions is extremely important in saving lives. Blood drawn from a suitable donor is treated usually with sodium citrate (which binds the calcium ions) to prevent coagulation. Care must be taken to prevent infection and the blood must be introduced into the recipient's veins at the proper temperature and rate. The heart will be overloaded if blood is transfused too rapidly. Blood can be stored for weeks in a "blood bank" by adding citric acid and glucose and keeping the blood at a temperature of 4° to 6°. C. By separating the red cells from the plasma and then suspending the cells in purified albumin, cells can be kept for as much as three months.

The search for substitutes for whole blood to be used in transfusions dates back to 1878. Plasma and certain fractions, which can be stored much longer than whole blood are effective substitutes for whole blood in many clinical conditions such as shocks Dried plasma and plasma fractions prepared by freezing and dry ing and placed in a sealed sterile container can be kept even without refrigeration for a long time. To be used, the plasma is mixed with proper quantity of sterile water and injected. In preparing plasma, bloods from sixteen different people of assorted blood types are pooled so that the different agglutinins are diluted below their effective concentration and will not agglutinat the red cells of the recipient.

The polysaccharide dextran, a glucose polymer made by bacteria can also be used as a blood substitute. It can be prepared inexpensively in large amounts, does not cause agglutination of red cells, gives fewer toxic reactions than any other substitute tried and eliminates the possibility always present in the transfusion of blood or plasma of trasmitting the virus of serum

hepatitis. Gelatin, pectin, gums and albumin from cow's blood have all been tried as substitutes but none has been satisfactory.

Rh FACTOR

In addition to the ABO system many other blood groups are now recognised. These include the Rhesus system, so called because it was first discovered by injecting rabbits with red cells obtained from the Rhesus monkey.

The majority of people possess RBC containing an antigen called the Rhesus factor. Their blood is described as RH positive. Those who lack Rh antigen are called Rh negative. Unlike the ABO system, Rh negative blood does not automatically contain the Rh antibody. However, if Rh positive blood finds its way into a Rh negative recipient, the latter responds by producing the corresponding Rh antibody. Nothing further happens; but if the Rh negative recipient subsequently receives another dose of Rh positive blood, these Rh antibodies will cause agglutination of the donors red cells, often with fatal results.

PROBLEMS IN PREGNANCY

Problems arise in babies born to Rh^-mothers and Rh^+ fathers. The foetus will be Rh^+. The Rh antigen in the foetus and blood cannot get across the placental barrier into the mother's

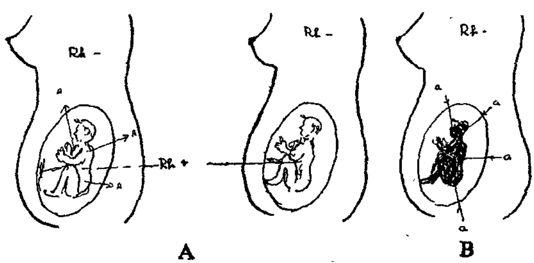


Fig. 178

Diagram of the sequence of events leading to Erythroblastosis foetalis, the clumping of red cells of the foetus within the uterus.

A. Just before birth, Red cells pass from an Rh+foetus to its Rh—mother through placenta, and maternal white cells produce anti Rh+antibody. The mother retains antibodies against Rh.

B. In subsequent pregnencies, mother's antibodies destroy baby's red blood cells.

blood during pregnancy, but can, at the time of birth. Then, the mother's immunity system mobilizes lymphocytes into immunocytes that produce anti-bodies against the Rh antigen. Since this occurs after the birth of the first baby, the first born is not usually affected unless the mother has already had antibodies against Rh produced from previous exposure to Rh antigens in injections or transfusions. However the second born will receive antibodies against Rh from the mother across the placental barrier before birth.

These antibodies clump RBCs of the foetus and can cause either mild symptoms or severe interference with circulation and the baby may die before birth or survive and suffer jaundice and liver failure. Occasionally the baby will be deaf or mentally retarded. The condition in the foetus brought about by the Rh incompatibility is called erythroblastosis foetalis and may be fatal unless the child's blood is replaced by transfusion with Rh- blood. Nowadays such transfusions are common practice and can be carried out while the foetus is still in the womb. It has recently been found that hemolytic disease can be prevented by treating the mother with an anti Rh-globulin that coats the foetal cells, thus blocking the Rh factor.

Genetics of Rh

The Rh protein is inherited as a result of 3 pairs of genes called C, D and E. But since the D gene seems to be more important, we will simply say that Rh is caused by one gene with 2 alleles, Rh positive and Rh negative.

Distribution

Rh negative is highest in W. Europe and rare in Asia. About 85% of white people are Rh positive.

LINKAGE AND CROSSING OVER

Soon after the rediscovery of Mendel's work, many scientists carried out experiments on various animals and plants. Very soon they found that Mendel's laws did not apply to all the animals and plants. In fact, he was lucky in his choice of the pea for his experiments, for, the traits he had chosen showed simple types of inheritance pattern.

In 1903, Sutton outlined the theory that Chromosomes are he bearers of the units of heredity and that each chromosome pair contained many such units called genes. Further he postulated that all such genes are linked together. Therefore, each species will have specific number of linked groups of genes which will correspond with the number of chromosome pairs.

Bateson & Punnet, two English geneticists were able to demonstrate the existence of such linkage and to prove that Mendel's law of independent assortment is not universally applicable.

The experiment conducted by Bateson and Punnet on sweet pea plant was as follows:

Plants with red flowers and spherical pollen grains were crossed with plants having purple flowers and cylindrical pollen grains The hybrids of F1 generation were backcrossed with plants having purple flowers and cylindrical pollen grains. Instead of the expected 1:1:1:1 ratio, 7:1:1:7 ratio was obtained. It showed that independent assortment as in dihybrid inheritence did not take place fully. Bateson & Punnet concluded that the free recombination was prevented because of the fact that the genes were linked. There is some recombination however, but it is less frequent than was found for non -linked genes.

Similar experiments conducted on Drossophila by Morgan confirmed the linkage of genes and recombinations which may occur in the linked genes at the time of gamete formation by crossing over; or exchange of genes between parental genes. When this happens, new combinations arise and they form the raw materials on which evolutionary process can work.

The frequency of crossing over between two groups of genes depends for the most part upon the distance between genes on the chromosome. In general, the farther apart two genes lie, the greater the percentage of crossing over, whereas two genes that lie nearer each other have less opportunity for crossing-over. A cross over in a particular region tends to inhibit the occurrence of another cross over nearby through a process called interference. Possibly this is to prevent a recross over and thus eliminate the results of the original cross over.

Many of the hundreds of genes so far discovered in Drosophila have been mapped out this way. A start has been made in mapping human genes.

There are other phenomena by which the genes in a chromosome become rearranged. Forexample, (1) a gene may be dropped out i.e. A B C D E F (genes on a chromosome) becomes A–B–D–E–F. This is called deficiency or deletion (2) a gene may be duplicated i.e. A B C D E F becomes A B B C D E F (duplication) (3) Part of the gene sequence is turned around *i.e.* A B C D E F becomes A B E D C F (Inversion) (4) A chromosome exchanges parts with one belonging to another pair and not with its own mate. For example A B C D E F and G H I J K L become A B C J K L and G H I D E F (translocation).

In inversions and translocations the same genes are present but their positions are changed. Sometimes no difference in phenotype is seen even though inversion or translocation may be present. The change in positions does affect the way the chromosomes pair in meiosis and this may cause a decreasd fertility or various peculiarities in inheritance. Sometimes the effect of a gene is changed simply because it has new neighbours. In Drosophila, for example, it has been found that size of the eye is affected by the genes which are next to each other. This is called **position effect.**

MUTATION

Hugo De Vries (1901) was the first to suggest that there is a possibiltiy of new types of inherited characteristics appearing suddenly without any previous indication of their presence in the race. He came to this conclusion from the experiments conducted on the evening primrose, Oenothera lamarckiana. Since his discovery, it has been found that most of the variations he observed were due to rare cross overs between translocated chromosomes rather than to changes in the genes. Yet, De Vries deserves credit for the concept of mutation since he was the first to suggest it.

Thomas Hunt Morgan (1910) made a scientific study of true-mutation in Drosophila. He discovered a few white eyed Drosophila among the normal redeyed ones. By crossing this 'mutanst' he was able to obtain a pure-breeding race of white eyed flies. This

change in eyecolor proved to be produced by a single gene mutation which had occured in an x-chromosome of a germ cell of the normal fly. Further scrutiny showed many similar mutations in Drosophila. The discovery of these made possible the extensive genetic studies on the fly.

Nature of mutations

The term mutation is often loosely used to denote all types of changes which result in a changed pattern of heredity. Two types of mutations are distinguished.

1. **Gene mutations** or point mutations which involve change in the genes.

2. **Chromosome mutations** or chromosomal aberrations which involve larger portions of the chromosomes (e.g.) deletion, inversion, translocation and duplication.

Gene Mutations

(1) Gene mutations occur suddenly.

(2) A mutated gene will be propagated through future generations although there may be rare instances in which it reverts to normal. Thus the mutations are not permanent.

3. Gene Mutations play an important part in evolution in that they provide new characteristics upon which natural selection can operate

4. Gene mutations can appear when there are small rearrangements or substitutions of the bases in the DNA molecule.

5. The gene is stable. H.J. Muller estimated that there is an average chance of less than one in a million of any given gene undergoing a mutation in Drosophila from the time it is formed by a replication of genes. It must be remembered how ever that many mutations do not show visible effects and the number of chromosomes in Drsophila are only 4 pairs. Thus it can be estimated that there will be some sort of mutuation for every 20 germ cells. However, all genes do not mutate with equal frequency.

6. Temperature and age influence the frequency of mutations.

7. Many mutations result in physiological changes with no recognizable effect on the body. For example mutation may affect fertility; most of them are lethal and there are also harmful

mutations such as albinism, aniridia (defective iris in the eye) alkaptoneuria, amaurotic idiocy etc.

8. A living organism is highly efficient but not perfect. Mutations represent random changes in the controlling mechanism of the living organism. It may be beneficial or not. The few beneficial changes which do occur provide a sufficient basis for evolutionary advance. Through selection, the many harmful mutations are held in check and eliminated, while the advantage held by the organism with beneficial mutation becomes established.

9. Mutations may not be essential to the survival of the species in certain limited conditions, but for most forms of life, mutations are essential.

10. Mutations may occur in germ cells, as well as in somatic cells. Cancer may be related to somatic mutations.

Induced Mutations

Mutations are valuable to the plant and animal breeder for improving his stock. It is also valuable to the experimental geneticist, for genes can be recognized and studied when mutations occur.

H.J. Muller was the first to show the effect of X-rays on Drosophila, in speeding up the mutation rate. Since that time X-rays and other sources of radiation have become standard methods of inducing mutation. Muller was awarded Nobel Prize in 1946 for his brilliant work in this field. Other sources of high energy radiation were tested for their mutagenic property. It was found that the radiation from radioactive isotopes of chemical elements would also increase mutation rate. Other agents such as ultraviolet rays, heat, chemical agents like nitrous acid, formaldehyde, ethyl-urethane, nitrogen, mustard, phenol, manganous chloride, bromouracil and even caffeine and theobromine can cause mutation.

Chromosomal aberrations can also be induced by radiation. Single break aberrations are usually lethal in their effects. Chromosome aberrations may result in phenotypic effects which are visible in the first generation after their occurrence, where as most gene mutations are recessive and are not easily detectable.

Chromosome aberrations are the cause of sterility in those exposed to ionizing radiations. Gene mutations may also be involved in some cases. Observations in Hiroshima indicate that many of the inhabitants of this city who received rather extensive radiation had no children for several years.

When a person is exposed to rather heavy but sublethal dose of radiation, certain parts of the body are affected much more severely than others. For example, the skin may become reddened, anaemia may develop due to reduced red blood cells etc. The actively growing and dividing cells are more sensitive to radiations.

The Cause of spontaneous gene mutations

Natural radiations from rocks, radiations in the food water and air taken in by animals or water, minerals and CO_2 taken in by plants, may cause the naturally occuring genetic changes. Other forces supplement this action. There are fluctuations of energy within molecules, and it is possible that a concentration of this energy on one bond of the DNA may cause breaks and rearrangements which could be mutations. Older parents transmit a slightly greater number of mutations to their off spring than younger parents.

CHAPTER XXIX

Sex Determination

For centuries, man did not know how the sex of the offspring is determined. However in the present century, knowledge as to the mechanism involved in determining the sex of the offspring has developed enormously.

In the late 1890's, scientists were beginning to establish the number of chromosomes in the cells of organisms. As a a matter of regularity, all sexually reproducing organisms had in their cells, a diploid number of chromosomes (referred to as 2n). In some insects, it was found that apart from the chromosome pairs an extra chromosome was also present and that too only in the males. This unpaired chromosome was named X chromosome. All the other chromosomes were called autosomes. Then it was discovered that the extra X chromosome had something to do with sex determination.

Today we know that atleast 5 different sex determination mechanisms are there in the animal kingdom. They are 1) Haploid-diploid type 2) XO type 3) XY type 4) WZ type and 5) the non chromosomal type.

The Haploid Diploid Type : During the production of gametes in sexually reproducing animals, the chromosome number is reduced to one half of what it is in the parent cell. (i.e. a diploid cell gives rise to haploid gametes). During fertilisation when 2 gametes meet the original diploid condition is restored. In honeybee, ants and and wasp fertilised diploid cell develops into a normal female. If the egg cell is not fertilised it grows into a male. Thus the males are haploid and females are diploid. The males produce gametes by mitosis only so that the male cells are haploid. The female produces gametes by meiosis, creating haploid egg cells.

The XO Type: In grasshoppers and in some insects the males have a set of diploid autosomes plus only one X chromosome. The females have a set of autosomes and 2X chromosomes. The female produces egg cells, all of which have a haploid set of autosomes and one X chromosome. The male produces sperms, half of which have a haploid set of autosomes, only and the other half have a haploid set of autosomes plus the extra X chromosome. Fertilisation results in 2 types of zygotes. One set with 2 X chromosomes forming a female and another set with only 1 X chromosome forming a male.

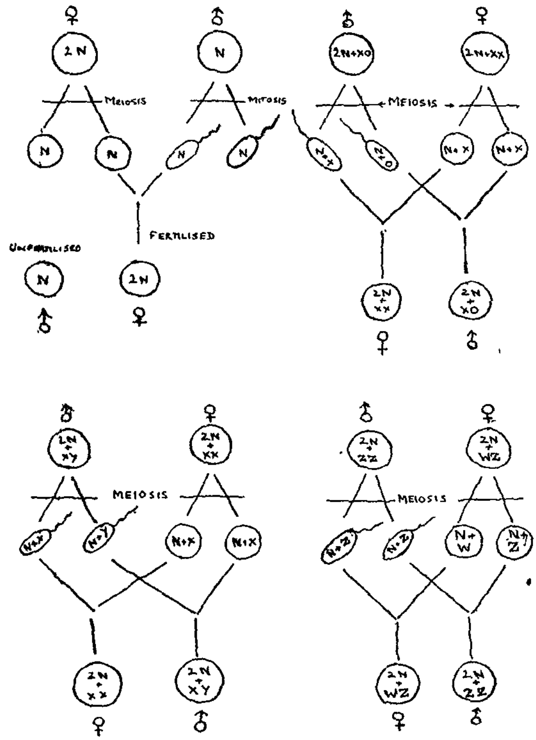


Fig. 177
Sex determination

The XY Type: In Man, some mammals and insects like Drosophila, the body cells of the male have a set of autosomes plus one X chromosome and another Y chromosome. This may

be slightly different from the X chroromosome and carries very few genes. During spermatogenesis the males produce two types of sperms, one type with an X chromosome and another type with a Y chromosome. But the female which has two X chromosomes besides autosomes,produces similar eggcells, all of which have one X chromosome besides a haploid set of autosomes. Depending upon whether an X bearing sperm or Y bearing sperm that fertilises the egg cell, the sex of the offspring is determined. If an X chromosome bearing sperm fertilises the egg cell than the zygote is XX and a female. On the other hand if a Y bearing sperm fertilises the egg-cell than the zygote is XY and a male.

The WZ Type: This is just opposite to the type described above. The female produces 2 types of gametes while the male produces similar gametes. For convenience we can say that females are ZW and their gametes are either Z bearing or W bearing besides autosomes. The males are ZZ and all the gametes are Z bearing. The zygote that becomesZW becomes the female and that which is ZZ becomes the male. This type is found in birds, butterflies etc.

The Non-chromosomal Type: In some animals the sex determination is not dependent on chromosomes. Environment and physiological conditions seem to determine the sex. Some molluscs are males but as they grow older they become females.

One interesting case of sex determination is that of a marine worm called Bonellia. The female is a bigger worm with a long proboscis. The male is very microscopic and lives in the genital duct of the females. If the zygote settles down independently it grows into a female. If it touches a female worm, then it grows into the demunitive male worm. Hormone from the female's body seems to interfere with sex determination.

At the first glance, it appears that the heterosomes or the sex chromosomes play the decisive role in determination of sex. But later researches have proved that they are not the only factors. The proportion between the autosomes and heterosomes or the ratio of sets of autosomes to X chromosomes determine he sex of the individual.

SEX LINKED INHERITANCE

Some characters are more commonly associated with a particular sex. The mechanism of sex determination by chromosomes explains this phenomenon. There are certain genes located on the sex chromosomes. The characters controlled by these genes are known as sex-linked characters. The inheritance of these characters is in different patterns according to the position of the genes in the X or the Y chromosomes. There are 4 main types of sex linked inheritance. They are :

1. Diagenic—The character passes from male to male through the female offspring.

2. Diandric—The character passes from female to female through a male offspring.

3. Hologenic—The character passes from female to female.

4. Holandric—The character controlled by a gene on the Y chromosome passes from male to male.

Many sex linked characters are known in Man. Some of them are colour blindness, haemophilia, night blindness, hypertrichosis, lcthyosis hystrix etc.

CHAPTER XXX

Inbreeding, Outbreeding and Heterosis

Since the beginning of civilization, man has been cultivating plants and breeding domestic animals, and by selection has tried to improve the quality of commercial types. Through applying the principles of genetics, he has already achieved remarkable progress in this field.

Inbreeding and outbreeding are two methods of breeding techniques. The mating of two closely related individuals is called inbreeding and the mating of individuals of totally unrelated strains is called outbreeding.

Inbreeding

It is commonly believed that inbreeding is harmful; but inbreeding is not always harmful, neither does it create harmful characteristics. In the lower forms of life, the closest form of inbreeding is self fertilization. This is possible only in hermaphrodites. Some plants avoid self fertilization by special adaptations such as ripening of the pollen and ovules at different times or through special devices for securing cross pollination. Other plants such as pea, bean etc. are habitually self pollinating and they do not lose their vigour by doing so. The best breeds of horses and dogs are usually, closely interbred. Thus, inbreeding is not always harmful.

Genetic explanation of the effects of inbreeding

Harmful characteristics often do become eivdent in inbreeding. This can be genetically explained. Most genes which produce harmful characteristics are recessive, and any individual is likely to be heterozygous for many of these. $\left(\frac{i}{+}\right)$ Such heterozygous parents will produce offspring in the simple Mendelian ratio of one normal $\left(\frac{+}{+}\right)$, two hybrids $\left(\frac{i}{+}\right)$ and one double

recessive $\left(\frac{i}{i}\right)$. Thus the double recessive character, whether harmful or not, can be expressed only if the parents are hybrids. This fact holds good, whether or not the parents are close relatives. In close breeding like brother-sister mating, the chance for both the parents being hybrids is greater and so the appearance of doudle recessives in the progeny is also more common.

Thus, inbreeding promotes homozygosity and eliminates hybrids. When this happens, purelines are established. Let us take, for example a population of 800 four o' clock plants, (Hybrids). Let us presume that one plant produces one offspring, so as to keep the number of plants constant. In such a population, F2 generation will produce 200 red 400 pink and 200 white flowers (1:2:1). The hybrids thus form only 50% of the total population. In F3 therse will be 200 pure line reds, 200 pure line whites and from the self pollination of 400 hybrids, 100 will be red, 100 will be white and 200 will be pink. The percentage of hybrids is thus reduced to 25%. The results are summarised below:-

Genaration	Composition of the Population			Hybrids	
	Reds	Pinks	Whites	Number	%
F_1		800		800	100%
F_2	200	400	200	400	50%
F_3	200		200		
	100	200	100	200	25%

Percentages of homozygosis compared with the original degree of heterozygosis has been calculated under different systems of inbreeding. In a population containing 50% heterozygous genes, homozygosity can be obtained within 8 generations

by self fertilization, in 16 generations in brother-sister or parent-offspring crosses. In mating between persons further removed than first cousins, increase in homozygosity is less pronounced. These calculations are very helpful for commercial breeding.

The development of homozygosity is influenced by factors such as linkage, procedures of selection and spontaneous mutation.

Descendants of inbred stock often show decrease in vigour. size and fertility. This can be counterbalanced to some extent by combining inbreeding with a programme of selection among the offspring so as to eliminate the harmful effects of homozygous harmful recessive genes.

Practical applications of Inbreeding in domestic animals

By controlling the matings of the animals within the hedr or flock and by selecting desirable traits, the breeder can develop a desribale genotype in the group. By guarding against introduction of genes from outsiders, he can stabilize the type. By this method, many valuable breeds of domestic animals have been obtained. Merino sheep of Spain is one such product of selective inbreeding, selected for fine wool. Pedigree horses and dogs are further examples.

To summarise, inbreeding does not allow only bad qualities to express themselves but any quality that is dependent on recessive genes would come to the surface. Inbreeding itself does not produce bad qualities. If a recessive gene is very common in a population, the chances for the parents to be hybrids are more, than if the recessive gene is rare in a population. The percentage of recessive genes in a population is not affected by inbreeding or outbreeding. Inbreeding tends to eliminate hybrids from a population and substitutes the pure types for them.

Outbreeding and Heterosis

Outbreeding is the mating of individuals of totally unrelated strains and it leads to offspring which are much better adapted for survival than either parent, a phenomenon termed **hybrid vigour** or **heterosis.**

Genetic explanation of heterosis

The biological basis for heterosis is unknown. Several hypotheses have been presented. One explanation of hybrid vigour lies in the fact that most harmful genes are recessive and most beneficial genes are dominant. Any hybridization tends to bring out the dominant qualitites to the surface. Such genes brought maximum expression in F1.

Let us consider an example, with 2 pairs of genes–gene A for improved root system and gene B for better chlorophyll system. Through inbreeding, each inbred line has become homozygous for its dominant gene. Thus a cross between two line of genotypes Ab/Ab and aB/aB will produce a hybrid of genotype Ab/aB which will show both improved root system and better chlorophyll system. On the other hand, if the inbred parents are AB/AB and ab/ab, the hybrid will be AB/ab and this will be no more vigorous than the parent AB/AB.

The genetic basis of heterosis is probably more complex than the above theory. Hybrid vigour is not usually due to hybridity itself but due to the fact that hybrids often contain dominant alleles for vigour at more loci than either parent.

In corn, it has been shown that there will be a gradual decline in vigour and yield for about seven generations, if seeds from a hybrid and its descendants are planted successively through this period of time.

Hybrid vigour may also be associated with polyploid condition. This is particularly common in grasses and is responsible for the ability of certain species to survive in arctic and desert conditions.

Practical applications of outbreeding

Heterosis has not been exploited in animals as in plants. Poultry and rabbit respond well to outbreeding. It has been effectively employed in the development of cattle. It is also used for the purpose of creating new breeds. It is a tedious and expensive process however.

An extreme form of outbreeding is cross breeding-mating of individuals from entirely different races and species. Successful interspecific crosses are rare in domestic animals. The classical

example of cross breeding is the mule, the result of successful crossing between two different species, the horse and the donkey. In many ways the mule is superior to either parent, strong and sturdy, better suited for many tasks than the horse or donkey. The mule is usually sterile. Each new generation must be produced from new crosses between donkeys and horses. The zebu has been successfully crossed with European domestic cattle. Other crosses include: bison X domestic cattle, domestic cattle and yak, gayal X domestic cattle.

Outbreeding in plants is widely used. Products of outbreeding in maize (corn) are found to be far superior to the products of inbred varieties. This has been widely exploited in India also and is one of the most practical attainments of genetics so far.

Both inbreeding and outbreeding are involved in the production of hybrid corn. Hybrid seed is produced by crossing inbred strains which are developed by controlled pollination. Only the most desirable lines are kept during the several generations of inbreeding. The inbred strain are then crossed to produce hybrid seed which grow into larger plants and are more productive. A technique called double cross hybridization is widely practised in the production of superior corn, in which cross beetween two hybrids are made. This technique combines beneficial dominant genes of four varieties in the individual. Today such double cross hybridization of corn has been standardized and the production of hybrid seed corn is a major agricultural pursuit.

Several plant species other than maize have been found to display hybrid vigour. Hybrid sorghums offer much promise. Procedures for producing hybrids have been improved in onions and sugar beet.

Comparative merits of Inbreeding and Outbreeding

In general, inbreeding in organisms capable of cross breeding tends to stabilize the type and weaken the individuals due to the accumulation of deleterious recessive genes in homozygous condition. If inbreeding is continued over a long period of time in the same environment, inefficient or defective individuals will be weeded out by natural selection and the strain itself may be improved by the elimination of recessive genes.

In contrast, outbreeding decreases the constancy of the type but increases the vigour of the individual. This type of mating tends to keep gene pairs heterozygous and thus protects recessives from the forces of natural selection. Outbreeding provides heterozygosity and greater variations for natural selection to work on.

Inbreeding does not allow for the introduction of good mutations from outside strains; outbreeding permits all good mutations that arise among the scattered members of a race to be concentrated into a single line.

For the speedy evolution of a race a combination of the two methods is desirable, viz., inbreeding for the most part interrupted by occassional outbreeding. In most species this normally occurs.